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EDITOR'S VIEWPOINT

Charter, Schlack, and Ware (p. 337) investigated procaine sensitivity among 3,951 Navy dentists by questionnaire method. They found 3 per cent professing sensitivity. Analysis of these 125 individuals' replies showed them to be older than the whole group and more regular users of self-prepared solutions. The reaction was usually dermatitis (85 per cent), with urticaria much less frequent. Only ten of the entire group indicated a disabling reaction within the prior fourteen-month period. Since procaine sensitivity is found in only a small group, and disabled only ten of 3,951 men, it might be well to weed out the procaine-sensitive individuals before admission to dental school and to encourage those who become sensitized during their early clinical experience to direct their interests toward specialization in fields not employing procaine.

Through a study of dental amalgams, which were mixed and packed mechanically, Phillips (p. 348) established a relationship between comparative strengths and time intervals following condensation. Each alloy rapidly gained strength during the first eight hours and then increased its strength slightly in the following days and months. The work reveals that the greatest danger of fracture is during the first few hours in the mouth. The safe time period for application of biting force on a well-constructed amalgam filling appeared to be eight hours, although the time interval may be shorter with faster setting alloys. The operator has a responsibility to properly warn the patient of the hazards of early biting on any newly placed amalgam restoration.

Irving, Weinmann, Schour, and Tweedy, in a series of experiments in calcification, first (p. 356) studied dentinal changes in the incisor of nephrectomized rats. They found the characteristic calciotraumatic band, an increase in dentin formation, and a few instances of ectopic calcifications in the pulp. A second phase of the study followed injections of large doses of calciferol in normal and nephrectomized rats (p. 362). In both groups, the calciotraumatic bands were found in the dentin. Calciferol increased the rate of dentin formation in normal rats but in the nephrectomized rats the injection of calciferol did not add to the increase in dentin formation observed in nephrectomized rats without calciferol treatment. In normal rats, calciferol increased the rate of calcification of dentin but this effect did not appear in nephrectomized rats. Injection of calciferol did not appear to influence ectopic calcification in the pulps. A third phase of these authors' study (p. 369) was on the effect of large doses of parathyroid extract on the dentin and pulp of normal and nephrectomized rats. Again the calciotraumatic effect was present as had been demonstrated after injection of fluorine, calciferol, and strontium, and following nephrectomy. This appears to be a nonspecific effect of interference with formation and calcification of dentin. Parathyroid extract decreased the dentin formation rate. Dentin resorption is not "a factor in the normal biology of dentin." Ectopic calcifications in the pulp were fairly regular findings following parathyroid extract injections, although less so than in the nephrectomized rats.

Besic (p. 379) when studying the action of acids upon extracted teeth observed lack of acid corrosion in areas of contact between teeth in solution and between the containers and tooth surfaces. A series of experiments was undertaken showing that the ions or molecules of a solution diffuse with difficulty into regions around contact points of immersed spherical bodies. The possible relation of this diffusion phenomenon to sites of predilection for dental caries is discussed. The prevalence of caries around contact points, within sulci, around clasps and fissures, and at gingival margins has long been associated with accumulation of debris and inaccessibility of these areas for cleaning. Besic's study strongly suggests an important role for diffusion about spheroid bodies in contact. A careful re-examination of common caries sites in relationship to such a phenomenon rather than to food accumulations is in order.

Hill and White (p. 391), from a study of acid production in saliva in vitro and in vivo, present the suggestion that factors other than the numbers of lactobacilli present may play an important role in acid production. We were impressed by the drop of the pH on the tongue of a caries-susceptible individual to pH 4.5 eight minutes after a twenty per cent glucose rinse. We had believed that the acid production in the free saliva would be buffered far above this range. Comparative studies of the pH in the plaque, and in the saliva at various points, certainly is in order.

Hill, Jelinek, and Blayney (p 398) made a survey which indicates that 5,646,897 individuals in the United States have access to communal water supplies containing 0.5 ppm of fluoride, while over 2 million are in the 0.9 to 2 ppm bracket and about ¾ million are exposed to more than 2 parts per million. The fluoride distribution in communal water supplies is of value in differentiating mottled enamel from other forms of enamel hypocalcification, in determining the advisability of topical application of sodium fluoride, and in determining the advisability of using fluoride-containing tablets (if they are shown to have any value). Surveys should be made at regular intervals to keep abreast of changing conditions.

Hadjimarkos and Storvick (p. 415) investigated the caries history of 741 children from two different geographic regions of Oregon. They found regional differences in caries experience. Bite-wing radiographic studies revealed 25.3 per cent more caries. We strongly suspect that variations in interpretations and care of clinical examination by different investigators and groups have produced even higher variations than this with apparently comparable methods. The most surprising finding was the complete absence of enamel hypoplasia in the 741 children.

Mitchell and Shafer (p. 424) began the feeding of caries-producing diets to hamsters at various prenatal and postnatal ages. In one series of experiments, animals receiving a caries-promoting diet at conception (via feeding to the mother) were in poorer physical condition and had more caries than those starting on the diet at weaning, but in another series, the findings were quite inconclusive. The authors found minute fracture-like lesions in the molars, and they open up a discussion of fracture-caries relationship suggestive of that in rat caries. Again the question of the relationship of caries in an experimental animal and in man must be raised.

Hurst, Mullett, Frisbie, Nuckolls, and Marshall (p. 430) in a progress report from California outline the difficulties of cultivating organisms from carious lesions. Experience with cultivation of surface (plaque) organisms would suggest that the efforts at sterilization of the surfaces lead to their negative results rather than that the organisms are "not cultivatable under the conditions used." The production of carieslike lesions by organisms in a media at neutral pH does not preclude production of acid which is neutralized by the material from the enamel and by buffering action of the media.

H. B. G. R.

A STUDY OF NOVOCAIN (PROCAINE) SENSITIVITY AMONG NAVY DENTAL OFFICERS

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INTRODUCTION

ANY sampling design has many variables that must be considered, some of which are common to sampling in general and others are related to the specific undertaking being considered. In the present study an attempt was made to obtain information concerning Dental Corps officers who have experienced allergic conditions attributed to the use of Novocain (procaine). No knowledge of the percentage of dentists in general who are sensitive to Novocain was available, and it was considered that this information was essential in making dental logistics studies in the Navy.§ It may be readily understood how this important problem might influence the personnel situation in any emergency that necessitated rapid mobilization. Furthermore, there were supplies to be considered and determinations to be made concerning the feasibility of using cartridge type syringes and other modern instruments that might eliminate or reduce the allergic symptoms. Since such purchases would be quite expensive, it may be seen that is was difficult to make proper decisions until the problem, itself, was circumscribed. If the manifestations were common and fairly serious, then the cost consideration would be subordinated to the potential gains or losses in manpower. If, on the other hand, the entire problem was found to be inconsequential and of little importance, then the negative findings might still be of major importance.

A questionnaire was developed for the specific purpose of attempting to evaluate the prevalence and seriousness of the Novocain-sensitivity experience. Approximately 7,500 forms were mailed to Dental Corps officers.

The evaluation of questionnaire utility immediately presents two questions that are very difficult to evaluate. One of these is related to the interpretation of the questions on the form and the influence that this variable has on the answers given. The other problem is associated with nonresponse or the questionnaires that were not returned. Every attempt was made to state the questions in a clear concise manner and to minimize the possibility of incorrect interpretations. In addition, some of the questions were stated in such a manner that made it possible to cross-check the answers of some of the other

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The opinions or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or the endorsement of the Navy Department.

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§This problem was originated and discussed by the Pharmacology Subsection of the Committee on Dentistry of the National Research Council, especially as it related to standards for entrance into the Navy Dental Corps.

The Council on Therapeutics of The American Dental Association has been making a similar study, and preliminary gross findings on Novocain sensitivity in civilian dentists have been somewhat similar.

questions. The questionnaire was framed in two parts. The first was a section on personal history and military status and the second was concerned with eliciting information relating to the conditions of sensitivity. The first part was completed by everyone; however, the second was answered by only the individuals who stated that they, at some time, had experienced symptoms of sensitivity to Novocain when handling it for administration to patients. It is very important to remember this fact because by using this procedure no information was obtained from those giving a negative answer to sensitivity concerning any preventive measures they may have used. In other words, the terms "sensitive" and "nonsensitive" are given to the two groups although it is not definitely known that the latter group is truly immune. It is possible that some of the individuals in the nonsensitive group may have escaped any troubles because they have always used some protection such as rubber gloves, protective cream, etc. While this is possible it is not very probable and it would not be particularly important in an evaluation of potential manpower. If a man were sensitive to Novocain and he was able, by the use of protective measures, to prevent the occurrences of allergic conditions he would be considered an effective individual. This is a two-sided problem and is also applicable to members included in the sensitive group. In the present survey the classification of a man as sensitive is based on his own evaluation of his condition. It is possible that some allergic manifestations were experienced concurrently with exposure to Novocain and mistakenly self-diagnosed as attacks induced by exposure to that drug.

DISCUSSION

As may be noted in Table I there were approximately 7,500 questionnaires mailed to Dental Corps personnel and of this number, 6,456 or about 85 per cent were sent to reserve officers not on active duty. It should be emphasized that the 7,500 is the total dental population of the Navy. However, it is obvious that the Navy population is in turn a sample of all of the practicing dentists. There is little reason to think that the experience of the Navy dental population differs greatly from that of civilian dentists since by far the greater proportion were civilian dentists who were on active duty only during the war period.

TABLE I
DISTRIBUTION OF THE QUERIES AND REPLIES BY MILITARY STATUS

MILITARY STATUS	NUMBER SENT	NUMBER RETURNED	PER CENT OF RESPONSE
U.S.N.	506	481	95
U.S.N.R., active	555	507	91
U.S.N.R., inactive	6,456	2,858	44
Total	7,517	3,951*	52

*Includes 105 military status unknown.

The over-all response of 52 per cent was extremely high and much better than the results ordinarily obtained in most questionnaire surveys. There were probably several reasons for this: (1) the recipients were a select group of professional people; (2) the tendency to regard any military request as an

order; (3) many of the individuals were interested in the subject and desired to assist in any professional research. There was a wide variation in the completeness of the response when the factor of military status was introduced. Ninety-five* per cent of the Regulars completed and returned their questionnaires as compared to a response of 48 per cent by the United States Naval Reserve group. However, the military influence can be more readily noticed when the reserves are divided into those on active duty and the inactive group. Ninety-one per cent of those on active duty returned their completed forms as contrasted to 44 per cent of the inactive Reserves.

In any consideration of the groups, as shown in Table II, it must be remembered that the individuals who stated that they had never experienced any allergic conditions when handling Novocain for administration to patients are called the nonsensitive group. On the other hand, all affirmative answers to the question, "Have you ever had symptoms of sensitivity to Novocain when handling it for administration to patients?" qualifed for inclusion in the sensitive group. It may be noted that if a person mentioned that he had a single symptom and it occurred twenty years ago he was still included in the sensitive group. Therefore, the total sensitive group is not a pure measure of the current problem. It is rather a measure of the current potential problem, if it is accepted that anyone ever having an attack is susceptible, under proper conditions, of having another.

TABLE II
DISTRIBUTION OF REPLIES BY MILITARY STATUS AND DECLARATION OF SENSITIVENESS TO NOVOCAIN

	MILITARY STATUS									
	NUMBER					PER CENT				
GROUP	TOTAL	U.S.N.	U.S.N.R. ACTIVE	U.S.N.R. INACTIVE	INFORMATION MISSING	TOTAL	U.S.N.	U.S.N.R. ACTIVE	U.S.N.R. INACTIVE	INFORMATION MISSING
All replies	3,951	481	507	2,858	105	100	100	100	100	100
Nonsensitive	3,823	465	494	2,760	104	97	97	97	97	1
Sensitive	128	16	13	98	1	3	3	3	3	99

Of the total of 3,951 replies, 128, or 3 per cent, stated that they had, at some time, experienced Novocain-sensitivity symptoms. This would seem to minimize the problem and to indicate that very few individuals are handicapped seriously by the administration of Novocain. However, here an evaluation of the nonresponses becomes an important factor. It is well known that individuals may be stimulated in either a positive or negative manner by questionnaire subject matter and by the phraseology of the questions. In the present study it is impossible to accurately gauge whether (and by what amount) sensitive people would be more or less likely to complete and return their questionnaires as contrasted with nonsensitive individuals. However, it is believed that people who had experienced any symptom of sensitivity would have been influenced to some degree by this event and that this force would have been exerted in a positive manner. If that were true, the returns would be

*There were 105 forms returned without information being recorded for military status, and probably some of these were received from individuals, both Regulars and Reserves, on active duty.

somewhat biased toward the sensitive group and the sample therefore would show a higher percentage of sensitive individuals than actually existed in the universe. Of course the reverse would be true if the ratio of sensitive to nonsensitive people were underreported in the sample. If the hypothesis stated above is true the returns provide a maximum parameter of the problem.*

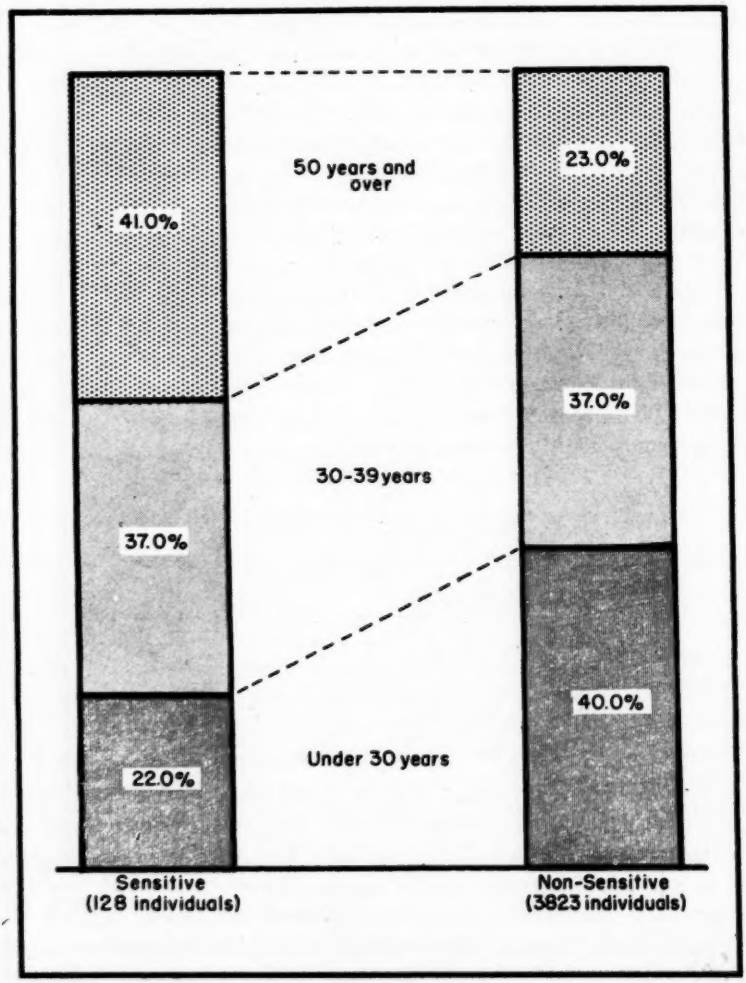

Fig. 1.—Comparison of the ages of nonsensitive individuals with those who have had Novocain-sensitivity symptoms.

The same percentage (3 per cent) of sensitive individuals was found among the Regulars and Reserves irrespective of whether or not the members of the latter group were on active duty. Of the 105 questionnaires received with military status unknown only one was recorded as a sensitive individual.

*Tables I and II indicate there was little bias in the reporting. The Reserve inactives with 40 per cent return showed the same (3 per cent) per cent positive as the Reserve actives with 90 per cent reporting.

Fig. 1 provides information relating to the difference in the ages of the two groups. While there is a difference in the mean age of the two groups,* the main interest is in the distribution of these ages. Twenty two per cent of the sensitive group were under 30 years of age as contrasted with 40 per cent of the nonsensitive group. The reversal was true for the older aged men where, as may be noted, 41 per cent of the sensitive individuals were 40 or over while only 23 per cent of the nonsensitive group were in this older age bracket. While these differences are significant, it is probable that they may be accounted for by definition rather than by a correlation of age and susceptibility to Novocain allergy. The question concerning this particular item, as was previously mentioned, was framed to obtain information as to whether an individual had ever experienced symptoms of sensitivity to Novocain. It is obvious that this is an open end variable whereby the older people have had a longer period of exposure, thereby increasing the probabilities of having had an attack. Furthermore, many of the younger dentists have only used some type of cartridge syringe which perhaps has provided some protection. Of the 48 individuals in the sensitive group who were 40 years of age or older, 14, or 29 per cent, did not have any attack after 1941. In contrast, there were only 7 or 16 per cent of the 30 to 39 age group who had their last attack prior to 1942.

In any evaluation of the sensitivity question it is important to attempt to estimate the seriousness of the problem to the Navy. This necessitates a review of the current situation together with an analysis of the possible or potential picture in case there is another mobilization. Table III provides a breakdown of the sensitive group by the year of last attack and military status.

TABLE III
DISTRIBUTION OF SENSITIVE GROUP BY MILITARY STATUS AND YEAR OF LAST ATTACK

YEAR OF LAST ATTACK	MILITARY STATUS			
	TOTAL	U.S.N.	U.S.N.R. ACTIVE	U.S.N.R. INACTIVE
1948	52	3	8	41
1947	14*	1	2	10
1946	6	1	–	5
1945	10	1	2	7
1944	5	–	–	5
1943	4	–	–	4
1942	5	–	1	4
Prior to 1942	22	8	–	14
Information missing	10	2	–	8
All cases	128	16	13	98

*Includes one military status unknown.

One of the difficult things to evaluate is the possibility of repeated attacks. Information was not obtained as to the frequency of the attacks nor as to the date of the origin of the symptoms. However, it would seem that if there had been a negative period of one year prior to the date of survey (February 1948) that the individual did not present a manpower-loss problem. With this interval in mind, Table III reveals the fact that only 66, or 56 per cent, of those for whom information was available experienced any sensitivity symptoms

*Sensitive Group $\bar{X}$ = 37 years. Nonsensitive Group $\bar{X}$ = 33 years.

during 1947 or the first two months of 1948. If the questionnaire returns are considered as being representative of the population, less than 2 per cent of the total Dental Corps personnel (7,517) experienced an attack during the fourteen-month period prior to the survey. In all probability part of this group of 66 men did not actually have an attack during this period as many of them (44) stated that they were presently sensitive although they did not state the specific year of their last symptoms. Of the 1,061 Dental Corps officers on active duty at the time of the survey, 29, or 2.7 per cent, gave affirmative answers to the sensitivity question and of this group 16 were Regulars and 13 were Reserves.* It is interesting to note the difference in distribution of the Regulars and Reserves on active duty by year of last attack. Only 4 of the 16 United States Navy group had an attack in 1947 or the first two months of 1948 while 10 of the 13 Reserves on active duty were classed as being positive for this period. On the other hand, 8 of the 14 Regulars for whom information was available stated that they experienced their last attack prior to 1942 while only one Reserve recorded his last symptom year as being prior to 1945. These differences partially substantiate the previous comments concerning the age factor and are probably explained by the fact that the mean age of the Regulars was 40 years and for the Reserves 32 years.

In order to try to better understand the problem under consideration it seemed desirable to attempt to ascertain what type of reaction the sensitive individuals experienced. This information is included in Table IV.

TABLE IV

DISTRIBUTION OF SENSITIVE GROUP BY TYPE OF SYMPTOM AND YEAR OF LAST ATTACK

PERIOD OF LAST ATTACK	TYPE OF SYMPTOM					
	TOTAL	DERMATITIS	URTICARIA	BOTH	OTHER	INFORMATION MISSING
1948-1947	66	56	5	1	3	1
1946-1942	30	26	3	–	1	–
Prior to 1942	22	18	–	1	3	–
Information missing	10	5	–	–	–	5
All Cases	128	105	8	2	7	6

It is interesting to note that 105, or about 85 per cent, of the individuals submitting complete information specified that the reaction took the form of a dermatitis while only 10 mentioned that they had urticaria, a more generalized condition. This latter group included two individuals who stated that their symptoms included both dermatitis and urticaria. Although the type of symptom gives some indication of the severity, it is difficult to make accurate statements on such a basis. While dermatitis ordinarily is not considered very serious, there is of course a range of these conditions from the very mild to the quite severe. Therefore, in order to obtain a clearer understanding of the problem, Table V is presented and the data, as shown in this table, present additional evidence concerning the severity and frequency of the symptoms.

*Since the return rates for Regulars and Reserves on active duty were 95 and 91 per cent respectively, it is considered unlikely that many sensitive individuals failed to return the questionnaire.

TABLE V

DISTRIBUTION OF SENSITIVE GROUP BY YEAR OF LAST ATTACK AND SEVERITY AND FREQUENCY OF SYMPTOMS

PERIOD OF LAST ATTACK	SEVERITY AND FREQUENCY OF SYMPTOMS									
	TOTAL*		NO DISABILITY†		PARTIAL DISABILITY		TOTAL DISABILITY		ATTACKS VARY IN INTENSITY	
	REG.	OCC.	REG.	OCC.	REG.	OCC.	REG.	OCC.	REG.	OCC.
1948-1947	30	35	20	24	9	8	1	–	–	3
1946-1942	7	23	5	19	1	4	1	–	–	–
Prior to 1942	10	9	5	6	3	3	2	–	–	–
Information missing	2	4	2	4	–	–	–	–	–	–
All cases	49	71	32	53	13	15	4	–	–	3

*Eight cases not reporting frequency of attacks not included. Four of these stated that the attacks did not prevent them from continuing operative procedures, one mentioned that he was partially disabled and three made no comments concerning severity of symptoms.

†Inconvenienced but didn't prevent conducting operative procedures.

One of the most important items that stands out in this table is the fact that only 4 of the 120 individuals mentioned that they were totally disabled by the symptoms caused by the administration of Novocain. It should be noted that all 4 of these men stated that the attacks occurred regularly on use. Furthermore, in Table VI it may be noted that all 4 of these individuals experienced symptoms that lasted longer than one month. Since only one of these individuals said that an attack had occurred within the last year, their questionnaires were carefully scrutinized in order to ascertain what preventive measures were used. One had received x-ray treatments in 1937 and later wore rubber gloves while operating for approximately six years. Since that time (1943) he has administered Novocain to patients without any ill effects. The other three obtained relief only ofter discontinuing the use of Novocain.

Twenty-eight, or 23 per cent, of the 120 cases shown in Table V stated that they were partially disabled by the symptoms, and of these, 15 experienced the attacks occasionally and 13 regularly when Novocain was used. Of the 13, 9 had their last attack in 1947 or the first two months of 1948. If these men were considered unfit for full duty, this would still leave only a very small segment (10) of the sample who might have been considered a liability to the Navy in 1947. Even if it should be determined that Novocain sensitivity of this degree was inconsistent with full duty status, the effect on the dental officer procurement would be slight, since only 10 of the 4,000-odd responses indicated a disabling sensitivity reaction within the fourteen-month period.

Eighty-five reported that they were sensitive to the administration of Novocain although the symptoms were annoying rather than disabling. Of these 85 almost one-half, or 41, had their last attack prior to 1947. There were 32 who mentioned that they experienced the symptoms regularly when they came in contact with Novocain and 20 of these 32 are currently sensitive or had an attack in 1947. The influence of wartime conditions and material upon this problem was considerable and several notes on the questionnaires mentioned that the prepared Novocain was the cause of the trouble. Some of the men experienced their only attacks while in the Navy and some idea of this may be obtained from the fact that only 7 of the 30 having their last symptom between 1942 and 1946 (inclusive) experienced symptoms regularly.

Another factor to be considered in evaluating the importance of Novocain sensitiveness is the duration or the length of time the symptoms last. An individual whose symptoms occur infrequently and then disappear within a day or two would present no major problem, either as a potential loss of manpower or as a medical case. On the other hand, the group having symptoms regularly lasting days or even weeks would be composed of men who probably should not be doing operative work where they would come in contact with Novocain in any form.

TABLE VI

DISTRIBUTION OF THE SENSITIVE GROUP BY DURATION OF ATTACKS AND SEVERITY AND FREQUENCY OF SYMPTOMS

DURATION OF ATTACKS	SEVERITY AND FREQUENCY OF SYMPTOMS									
	TOTAL*		NO DISABILITY		PART DISABILITY		TOTAL DISABILITY		ATTACKS VARY IN INTENSITY	
	REG.	OCC.	REG.	OCC.	REG.	OCC.	REG.	OCC.	REG.	OCC.
Less than 1 week	18	34	15	33	3	1	–	–	–	–
1 week	6	15	4	9	2	6	–	–	–	–
2 weeks	7	6	5	3	2	1	–	–	–	2
4 weeks	7	11	2	5	2	6	3	–	–	–
Continuous with use	10	3	6	3	3	–	1	–	–	–
Information missing	1	2	–	–	1	1	–	–	–	1
All cases	49	71	32	53	13	15	4	–	–	3

*Eight cases with missing information concerning frequency of symptoms are not included.

Fifty-two, or 43 per cent, of the 120 individuals had (or have) symptoms that lasted less than one week. At the other extreme were the 13 men whose attacks lasted continuously while being exposed to Novocain. While the data are not susceptible to detailed technical analyses, there are some pertinent facts revealed in the above table. In the first place there seems to be a definite relationship between the severity and duration of the symptoms. For example, the individuals whose symptoms were nondisabling show a somewhat shorter duration time than the ones with more severe symptoms. Furthermore, the men having regular attacks upon exposure to Novocain tend to have a longer duration than those subjected to only occasional attacks. In all probability there is a definite correlation between the duration of the attacks and the severity and frequency of these symptoms.

Irrespective of the preceding comments, one of the most important tasks is to estimate the problem to the Navy and what the picture would have been if the sample population had been in service from the first of 1947 through February of 1948. This information is depicted in Fig. 2. There was a total of 66 individuals who stated that they were either currently sensitive or had experienced a sensitivity symptom during this period. This is 46* per cent of the (128) sensitive group and approximately 1.5 per cent of the total group returning the questionnaires. Of these 66 individuals, only one man stated that he was totally disabled during the course of his attacks and he further mentioned that he experienced symptoms continuously while being exposed to Novocain. Of the 65 for whom information was available, 30 said

*Seven individuals with information missing not included in Fig. 2. Six of these did not mention how long the symptoms lasted and one failed to comment regarding the frequency of the attacks.

that the symptoms appeared regularly with exposure and 35 had occasional attacks even though they were having continuous exposure. Twenty-seven of the thirty having regular symptoms gave the duration of the symptoms, and of these, 10 lasted less than one week with only 4 mentioning a period longer than a month. Five of these 27 recorded the fact that their symptoms con-

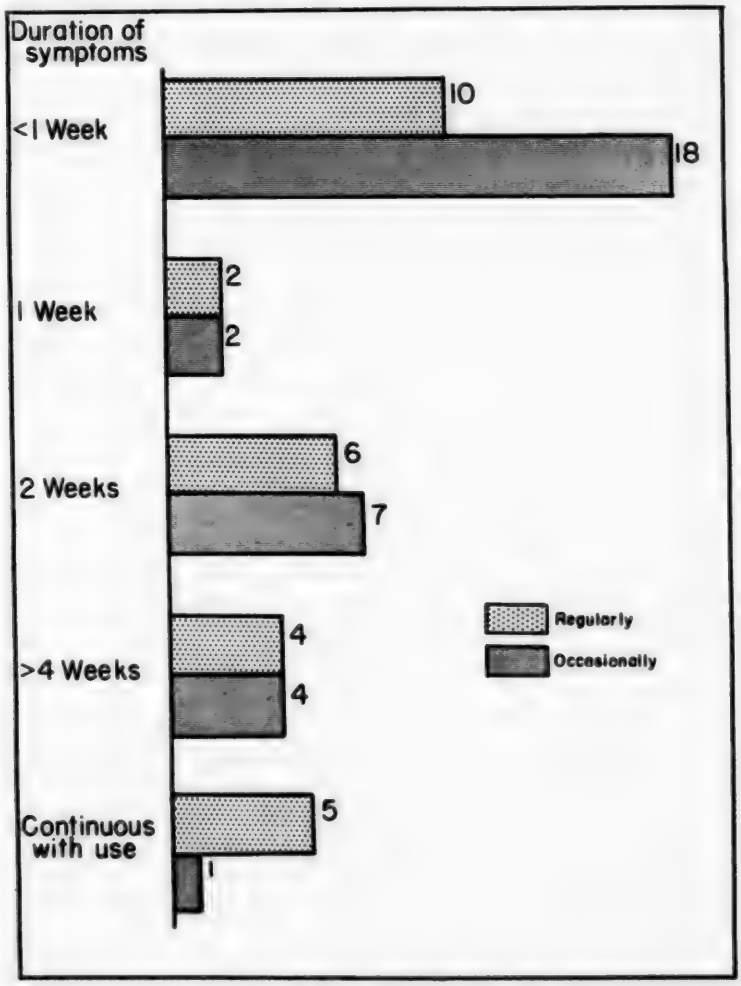

Fig. 2.—Distribution of the individuals having attacks during the fourteen-month period by duration of symptoms.

tinued until they stopped being exposed. From these observations it would appear that the manpower loss would have been minor if all of the respondents had been in the Navy and there had been no deviation from their actual experience.

One of the important factors in the administration of Novocain is the method of preparation and the type of container and syringe used in the administration. As was previously mentioned, the type of syringe used could

have been the determining factor in some individuals being included in the sensitive group and it is quite possible that others may be sensitive and never know it because they have never mixed the solution from tablets nor used the open type syringe. Age, location, economics, assignment, and perhaps many other determinants have influenced the individual use or non-use of commercially prepared Novocain cartridges or ampules, etc. Nevertheless, it was interesting to note that at the time of the attack 21 men were preparing the Novocain solution from tablets or crystals, 89 were using commercially prepared cartridges, while only 3 were using commercially prepared ampules and drawing the solution into the syringe from them. Four of the remaining 15 men did not comment concerning the method of preparation and the rest (11) were using combinations of types.

Since the majority of the men said that they were either preparing the Novocain solution from tablets or using commercially prepared cartridges, it might be well to make some additional comments concerning the frequency of the symptoms experienced, by the men in these two groups.

TYPE OF NOVOCAIN	REGULAR SYMPTOMS	OCCASIONAL SYMPTOMS
Self prepared*	13	6
Commercial cartridge†	29	57

*Two men not commenting on frequency of symptoms omitted.
†Three men not commenting on frequency of symptoms omitted.

From the above presentation is may be seen that the two groups, classified by type of Novocain used, show an interesting difference in the frequency of the attacks or symptoms. There is almost an inverse ratio between the two groups of the individuals having regular symptoms. Approximately two-thirds of the group preparing the Novocain from tablets or crystals experienced symptoms regularly upon exposure while only about one-third of those using commercially prepared cartridges were thus affected. Whether this difference is accounted for by the fact that one is much more apt to get Novocain on the hands if the solution is self prepared is unknown. However, this is a significant difference and it is unlikely that this is accounted for by chance.*

Various types of protection were tried by the sensitive individuals with varying degrees of relief. Of the 128 individuals, 89 tried one or more methods of protection with some measure of success. Twelve tried some method without finding anything that would help them while 4 stated that they had tried nothing whatsoever. Of the protective materials tried, rubber gloves and cream were the most popular, with the former the more effective. The largest number (92) attempted to avoid touching the solution, and of this group all but 20 reported that this method was partially effective in preventing the sensitivity symptoms. However, as one might expect, the only 100 per cent effective counter-measure that was tried was the step taken by 18 men who discontinued using Novocain.

One of the interesting conditions of people who exhibit an allergic manifestation is the relation to other sensitivity symptoms. For the 122 men that

*P = .01.

answered the specific question, 21, or approximately 17 per cent, stated that they were sensitive to some other local anesthetic solution.

SUMMARY

1. A questionnaire was developed for the purpose of attempting to evaluate the prevalence and seriousness of Novocain-sensitivity symptoms among Navy dental officers.

2. A total of 7,517 questionnaires were mailed to Regular and Reserve dental officers with an over-all response of 52 per cent. This response varied from 44 per cent for United States Naval Reserve inactive personnel, to 91 per cent for United States Naval Reserve (active), to 95 per cent for those classified as United States Navy.

3. Of the 3,951 replies received, 128, or 3 per cent, were grouped as sensitive. This 3 per cent was constant for all three military groups.

4. The sensitive individuals (as expected) were older than the members in the nonsensitive group. It is believed that this may be attributed to definition rather than a true relationship between age and the susceptibility to Novocain sensitivity.

5. Only 66 dental officers or 56 per cent of the sensitive group experienced any symptoms during the fourteen-month period prior to receiving the questionnaire. This included 44 who stated that they were currently sensitive although they did not state the specific year of their last attack.

6. Eighty-five of the sensitive group stated that the reaction took the form of a dermatitis while only 10 mentioned that they had urticaria. There were 7 who recorded some other type of symptom.

7. Only four individuals stated that they were kept from performing all operating procedures by the attacks although 49 mentioned that they experienced symptoms regularly upon exposure.

8. Fifty-two, or 43 per cent, of the 120 individuals for whom information was available had (or have) symptoms that lasted less than one week while 13 had attacks that lasted continously while being exposed to Novocain.

9. The men having regular attacks upon exposure to Novocain tend to have a longer duration than those subjected to only occasional attacks.

10. Of the individuals stating that they were either currently sensitive or had experienced Novocain-sensitivity symptoms in the previous fourteen-month period, 30 said their attack occurred regularly. Only 4 of the 30 mentioned that their symptoms lasted longer than one month and 5 recorded the fact that the symptoms continued as long as they were exposed.

11. Only 10 of the 4,000-odd dental officers returning the questionnaire indicated a disabling sensitivity reaction within the fourteen-month period.

12. There was a significant difference in the men experiencing symptoms regularly between those using self-prepared solution and those using commercially prepared cartridges.

13. The only entirely effective counter-measure that was tried was the step taken by 18 men who discontinued using Novocain.

COMPRESSIVE STRENGTH OF AMALGAM AS RELATED TO TIME

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THERE is no doubt that adequate crushing strength is essential to the success of the amalgam restoration. Fracture, even on a small area, will hasten deterioration, recurrence of decay, and subsequent clinical failure.

The requirement for compressive strength has been deleted from the American Dental Association Specification #1 because most amalgam alloys which pass the flow test will also meet the former crushing strength requirement of 35,000 pounds per square inch after twenty-four hours. However, daily observations in the dental office reveal many fractured amalgam restorations. A recent study at Indiana University School of Dentistry showed that approximately 26 per cent of the 1,526 amalgam failures examined failed due to fracture.[1] Since at the present time there are very few definitely inferior alloys on the market, these fractures had to be attributed to either (1) improper cavity preparation, which usually resulted in inadequate bulk of material, or to (2) faulty manipulation of the amalgam. The more important contributing factor was found to be faulty manipulation. Research has clearly shown that various manipulative factors, such as improper mercury-alloy ratio, undertrituration, and improper condensation, can markedly lower the crushing strength of amalgam.[2, 3, 4] Consequently, it must be constantly emphasized that not only is it necessary to have proper cavity preparation, but also an exacting manipulative procedure should be maintained to assure maximum strength.

Although most alloys may possess a high strength after twenty-four hours, it is quite possible that many of the fractures occur very shortly after the amalgam restoration has been completed. Since amalgam apparently gains its strength relatively slowly, many dentists caution patients against biting on the restoration during the first twenty-four hours. These men have felt that the maximum strength is not reached until that time and the amalgam would be subject to fracture during that interval. However, there are others who believe that amalgam gains its strength more rapidly than this, and they feel that the patient can bite safely on the restoration after the first few hours. It is a controversial issue and no research data are now available to indicate the strength of amalgam as it is related to time, although Taylor, Sweeney, and their co-workers, are at the present time conducting some studies along this line.[5] The purpose of this study was, therefore, to determine the crushing strength, and hardness of amalgam at various time intervals ranging from one-half hour after condensation to six months. Such data would be valuable

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not only in accurately establishing the safety period necessary before a biting stress can be applied to the freshly inserted amalgam restoration, but also in shedding some additional light upon the complex setting reactions of amalgam.

PROCEDURE

It was important in this investigation to control all of the manipulative variables which could alter the crushing strength. In order to compare strength of an alloy at various time intervals, the individual specimens had to be prepared in a uniform manner.

Six popular commercial alloys, purchased on the open market, were used, including small-, medium-, and coarse-grained alloys. It was felt that the results on these representative alloys would be indicative of what would occur with most present amalgams. In order to eliminate the possibility of variations in different batches of the same alloy, each one was purchased in large enough quantities that the same lot number could be used during the entire study. The recommended mercury-alloy ratio, weighed by a Crandall balance, was followed for each alloy and a constant-size mix was used throughout.

Since a slight under- or overtrituration will markedly alter the crushing strength, it was important that each mix have as nearly as possible the same consistency. In order to eliminate some of the human variables involved in hand trituration, all mixing was done by means of a mechanical amalgamator. Fluctuations in line voltage, which can alter the r.p.m. of the amalgamator, were controlled by means of a variable transformer. Once the mixing time had been properly established for each alloy, that particular timing was used on subsequent mixes.

It is also an established fact that the strength of amalgam is influenced by variations in the condensation pressure and packing procedure. Since it is most difficult, if not impossible, to control accurately the packing thrust either by hand or by mechanical condensation, all specimens in this investigation were prepared by means of an automatic packing device. No mercury was expressed after trituration, but the entire mix was immediately placed in the mold and a static load of 4,000 pounds per square inch was applied for two minutes. The specimen, 10 mm. by 5 mm., was then trimmed smooth with a razor blade, removed from the mold, and stored in air in an oven held at 37° C. ±0.5° until time for the crushing test. A Tinius Olsen machine was used for determining the crushing strength, the load being applied at the rate of 650 pounds per minute. At least five specimens were run for each time interval and any specimen which varied by more than 15 per cent from the average was omitted from the data. Crushing strengths are reported to the nearest ten pounds.

Hardness measurements were made by means of a Tukon Tester using the Knoop Indenter. A load of 200 grams was used.

It was felt that the foregoing technic would routinely produce specimens which were unaffected by human variables, such as trituration and condensation.

RESULTS

The data, showing the compressive strengths at various time intervals following condensation, are given in Table I. Each figure represents an average of at least five individual specimens. Alloys E and F were studied only for the first twenty-four hours, but data on alloys A, B, C, and D were also obtained on specimens which had been stored for forty-eight hours, one week, two weeks, one month, and six months. Crushing strength of these four alloys is plotted in the graph in Fig. 1.

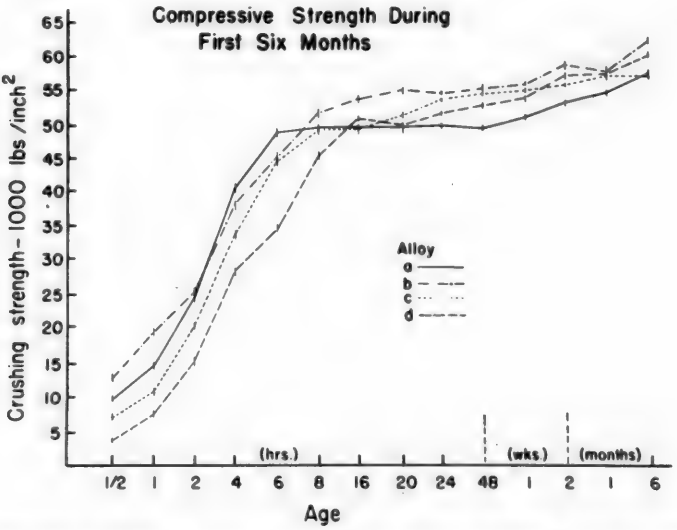

Fig. 1.—Compressive strength of four alloys at various ages, ranging from one-half hour to six months.

TABLE I
COMPRESSIVE STRENGTH OF SIX ALLOYS AT VARIOUS TIME INTERVALS*

TIME	CRUSHING STRENGTH—POUNDS PER SQUARE INCH					
	A	B	C	D	E	F
½ hour	9,570	12,420	6,990	3,650	10,870	7,500
1 hour	14,660	19,300	10,850	7,680	22,130	12,650
2 hours	24,610	25,230	19,880	15,280	36,910	23,910
4 hours	40,480	38,500	33,840	28,490	46,320	40,690
6 hours	48,750	45,360	44,850	34,440	-----	-----
8 hours	49,410	51,900	49,540	45,640	45,720	44,230
16 hours	49,560	53,770	49,770	51,100	50,180	46,150
20 hours	49,970	55,160	51,760	50,100	49,780	45,890
24 hours	50,050	54,480	53,780	51,830	51,540	45,960
48 hours	49,680	55,290	54,560	52,830	-----	-----
1 week	51,370	56,010	55,140	53,800	-----	-----
2 weeks	53,440	58,740	56,040	57,360	-----	-----
1 month	54,960	57,710	57,360	57,460	-----	-----
6 months	57,880	62,530	57,290	60,550	-----	-----

*Alloys E and F were tested only during the first twenty-four hours.

A study of these data and the graph indicates that each alloy gains compressive strength rapidly during the first eight hours. The strength then levels off with only a gradual, slight increase during the following hours, days, and months. When compared with the ultimate compressive strength obtained, all of the alloys do, of course, exhibit low strengths during the first few hours. However, it is apparent that some of the faster setting alloys gain in early crushing strength quite rapidly. For example, the difference in strength between alloy D, which is slow setting, and Alloy E, which is fast setting, is 14,450 pounds at the end of one hour; but, at the end of eight hours, Alloy E is only 80 pounds stronger (that figure being within experimental error). With the four alloys which were tested over a period of six months, the difference in compressive strength at the end of six months between the strongest (B) and the weakest (C) was only 5,240 pounds. Thus it appears that any greater difference between the compressive strengths of the various alloys, when handled by a controlled procedure, is evidenced only during the first few hours of setting. Factors such as particle size, composition, annealing process, etc., would determine the setting time and the resulting strength during these initial hours.

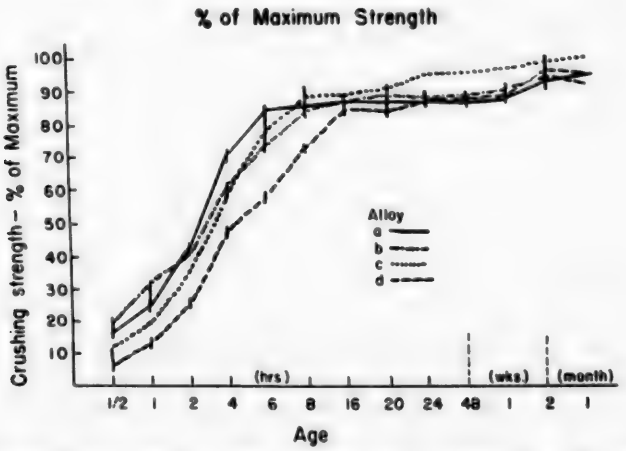

Fig. 2.—Per cent of maximum compressive strength plotted against age of the specimen.

The data for alloys A, B, C, and D were also tabulated in somewhat different form. Assuming the maximum compressive strength to be that figure reached at the end of six months, the strength at each particular time interval has been computed in terms of the per cent of this maximum strength.* For example, at the end of one-half hour, Alloy A had a crushing strength of 9,570 pounds which is 16 per cent of the maximum figure (57,880) reached at the end of six months. These data are shown in graphic form in Fig. 2 where the age of the specimen is plotted against the per cent of maximum strength attained. These curves emphasize more clearly the fact that all of the alloys gain their com-

*It is quite true that these alloys may not have reached maximum strength at the end of six months but may continue to increase gradually over a longer period. Additional long-range tests are now under way.

pressive strength rapidly during the first eight hours. At the end of this time, three of the alloys had attained over 80 per cent of their ultimate, final strength, but slower setting Alloy D had reached only 72 per cent of its maximum. (However, it should be noted in Table I that at the end of eight hours, the actual strength in terms of pounds per square inch of Alloy D was almost equal to that of Alloy C.) The curves do graphically illustrate how the alloys gain a very high per cent of their maximum strength during the first eight hours. The strength then levels off and there is only a gradual increase up to the maximum at the end of six months.

The results of the hardness tests closely parallel the crushing strength data. In order to eliminate the possibility of slight differences in individual specimens, hardness was measured on the same specimen throughout the six-month period. For example, the Knoop hardness at the end of six months was obtained on the same specimen which was used for measuring the one-hour hardness. The hardness number at each time interval was computed as the average of three individual specimens, with at least two indentations being made on each specimen

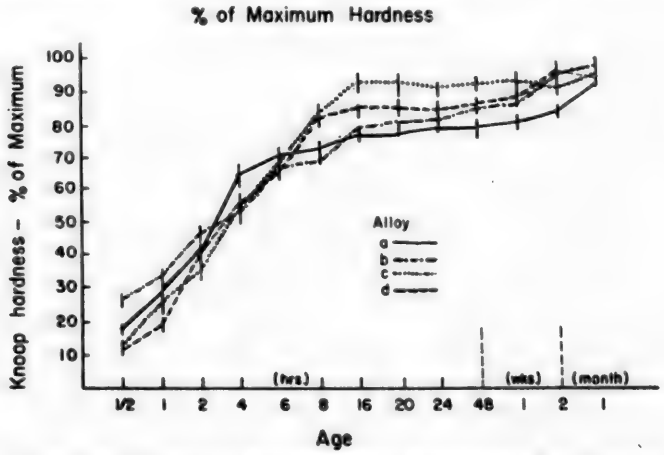

Fig. 3.—Per cent of maximum hardness plotted against age of the specimen.

The per cent of maximum hardness (that obtained at the end of six months) for each time interval was computed and is shown graphically in Fig. 3. Although most of the alloys have not reached as great a per cent of their maximum hardness at the end of eight hours as they did in crushing strength at that time, these data do indicate that generally the hardness shows the same trend as the crushing strength. The greater per cent of the maximum hardness is attained by the end of the first eight hours and is followed by a continuous, gradual increase up to six months.

The practical significance of these hardness data is questionable and probably serves only as a general check on the crushing strength measurements. As the two are interrelated, it would be anticipated that hardness would parallel the crushing strength.

During the course of this investigation, tests were also run in which the specimens were stored in synthetic saliva at 37° C. instead of in air at that temperature. It was felt that storage in saliva would more closely compare to clinical conditions. No difference was noted. Likewise, crushing strength was determined at the various time intervals on specimens which were packed by hand rather than by machine. Although the strengths were generally slightly less all the way through, results were similar.

DISCUSSION

This investigation is possibly of greater academic interest than of practical importance. However, it can be seen from these data that the real danger of fracture in the amalgam restoration lies during the first few hours after it has been placed in the mouth. The strength at that time, particularly with a slow setting alloy, is extremely low, for example, being only 7,680 pounds per square inch at the end of one hour for Alloy D. Other commercial alloys not tested here may have even less crushing strength at one hour. Wilful or accidental stress on the restoration at that time would no doubt cause fracture. Fortunately, the present amalgam alloys do gain their compressive strength rather rapidly and have, in general, attained approximately 85 per cent of their maximum strength by the end of the first eight hours.

It would be rather difficult to establish definitely the critical period and to determine at what point biting stress can safely be applied to the amalgam restoration. Unfortunately, we do not know what the desired minimum strength need be, and it is not the purpose of this paper to establish a specification or a requirement for crushing strength. Various factors play a part, not the least of which is the bulk of amalgam which is present. If there is adequate bulk, then the inherent strength of the alloy need not be so great. If proper cavity preparation is not maintained and a small isthmus is present, the strength must, of course, be greater in order to prevent fracture. For example, a biting stress of 100 pounds, which is not abnormal, placed on an area the size of a No. 558 bur would mean an actual compressive strength of 60,000 pounds per square inch on that area. None of the six alloys tested by the procedure described here had that much strength even after twenty-four hours. Thus, the minimum limit for compressive strength would vary for the bulk of amalgam present as well as for various other factors, such as testing procedure.

However, the findings presented here do indicate that for the properly prepared restoration, it would be safe to apply a biting stress after eight hours. Increase in strength after that point is not of great enough magnitude to warrant a longer waiting period. Of course, the faster setting alloys, for example, alloy A, would have a lower minimum safety time. This alloy had reached 84 per cent of its maximum strength after only six hours. It might even be argued by some that the strength attained at the end of four hours by most alloys is adequate to resist normal biting stress. However, to minimize the chances of early fracture with most alloys, it does appear that biting stress should not be applied during the first eight hours.

The results of this study indicate that the strength of amalgam closely parallels the setting reaction. This is illustrated in Fig. 4 where, on the same graph, have been plotted the crushing strength and dimensional change for Alloy A during the first twenty-four hours. During the first few hours, amalgam undergoes both a marked contraction and expansion. These dimensional changes can be explained on the basis of the chemistry of the setting reaction.[6, 7, 8] It is interesting to note that during this time the crushing strength is also increasing rapidly. Then approximately six hours after the start of the mix, as the formation of the crystalline $\gamma 1$ phase slows down, the dimensional change becomes stationary and likewise the strength levels off. This correlation would be anticipated, but it has not been previously demonstrated that the strength is directly related to the crystallization and setting reactions.

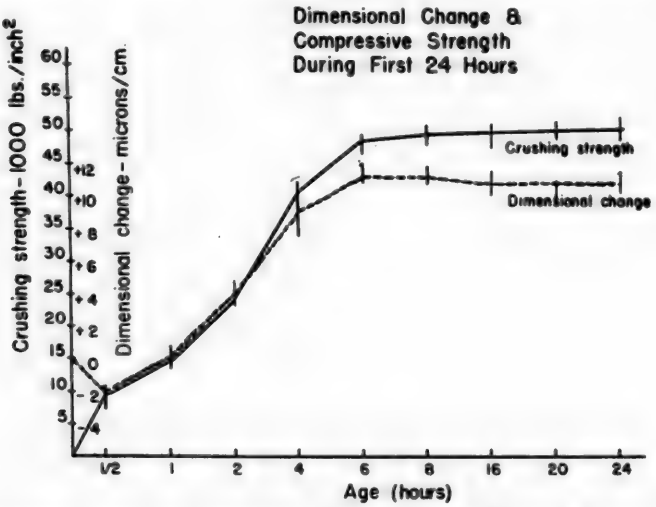

Fig. 4.—Dimensional change and compressive strength of Alloy A during the first twenty-four hours.

It is thus quite possible that the subsequent strength increase after twenty-four hours can be associated with continued chemical reactions. Gayler[9] and others have shown that the dimensional change fluctuates over a long period of time and, as pointed out by Skinner,[10] it is quite possible that equilibrium in the mouth is reached only after an indefinite period, if ever. The findings here that the strength does continue to increase over a long period of time seems to substantiate that belief.

CONCLUSION

Six popular alloys were studied in order to determine their compressive strength as it is related to time. Crushing strength was tested at various time intervals ranging from one-half hour up to six months.

It was found that amalgam, though quite weak during the first few hours, rapidly gains its strength and generally reaches approximately 85 per cent of its

maximum strength by the end of the first eight hours. The compressive strength thereafter continues to increase gradually up to the sixth-month final test. Hardness measurements verified these findings.

These crushing strength measurements can probably be associated with the setting reactions and indicate that equilibrium in amalgam is not reached for an indefinite period of time.

The author wishes to acknowledge the very capable assistance of Mr. James O. Stewart in this investigation.

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EXPERIMENTAL STUDIES IN CALCIFICATION

VII. Dentinal Changes in the Incisor of the Nephrectomized Rat

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INTRODUCTION

PREVIOUS investigations have shown that acute changes in mineral metabolism leave an imprint upon the dentin of the continuously growing rat incisor in the form of a traumatic band[1] of altered calcification. A thin layer of dentin which stains deeply with hematoxylin, the calciotraumatic line, separates the affected dentin from the normal dentin. The outer or older layer of the affected dentin, which retains an eosinophilic staining reaction, corresponds to the layer of uncalcified predentin that was present at the beginning of the experiment. It remains hypocalcified. The inner or younger layer, which is basophilic and is produced in the first twenty-four hours after the onset of the experiment, is hypercalcified. The boundary between the hypocalcified and the hypercalcified layer (the experimental dentin line[1]) is often sharp and divides the pre-experimental from the experimental dentin. Although the pattern of the dentinal change is always the same in principle, it shows in details characteristic differences in various experimental conditions.

The important role which the kidneys play in the regulation of the mineral metabolism is well known. Tweedy and McNamara[2] have shown that after nephrectomy the serum calcium remained within normal limits or fell gradually, while the serum inorganic phosphorus increased. The present investigation aims at establishing the changes in the dentin and in the pulp after bilateral nephrectomy. The literature shows no record of a comparable study.

METHODS AND MATERIALS

The study is based on an investigation of the upper incisor teeth of nine normal control and seventeen experimental white rats of the Wistar strain (Tables I and II). The animals varied in age and their weight ranged from 60 to 287 grams. The animals had been maintained on a diet of Fox Chow which was supplemented by meat scraps and cabbage leaf twice a week.

The experimental animals were subjected to bilateral nephrectomy under ether anesthesia and were then starved, being allowed water only. Two were sacrificed twenty-eight hours later and the rest after forty-eight hours. The control animals were also starved and allowed only water for forty-eight hours before being killed.

Immediately after sacrificing, the heads were removed and fixed in 10 per cent formalin for a few days and then decalcified in 5 per cent nitric acid. Central sections of the upper incisors were prepared and stained with hematoxylin-eosin.

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TABLE I

WIDTH OF PREDENTIN OF CONTROL ANIMALS STARVED FOR FORTY-EIGHT HOURS

NUMBER AND SEX OF ANIMAL	WEIGHT IN GRAMS	WIDTH OF PREDENTIN	
		AT FORMATIVE THIRD IN μ	AT MIDDLE THIRD IN μ
501 M	141	20.8	11.7
502 F	104	26.0	19.5
511 F	136	26.0	13.0
512 F	140	—	9.1
513 F	143	—	13.0
519 F	86	27.3	14.3
620 F	91	26.0	13.0
682 M	88	24.7	9.1
684 M	60	24.7	13.0
(Mean) Average		25.0 ± 1.01	12.8 ± .95
Range		20.8 – 27.3	9.1 – 19.5

TABLE II

EFFECT OF NEPHRECTOMY ON FORMATION OF DENTIN AND ECTOPIC CALCIFICATION OF THE PULP

NUMBER AND SEX OF ANIMAL	WEIGHT IN GRAMS	DURATION OF EXPERIMENT IN HOURS	WIDTH OF PREDENTIN		DISTANCE OF		
			AT FORMATIVE THIRD IN μ	AT MIDDLE THIRD IN μ	EXPERIMENTAL DENTIN LINE FROM PULP IN μ	CALCIOTRAUMATIC LINE FROM PULP IN μ	CALCIFICATION IN PULP
506 M	151	28	33.8	14.3	—	14.3	0
507 M	163	28	35.1	6.5	14	19.5	0
503	119	48	35.1	27.3	36.4	61.1	0
504 F	113	48	39.0	33.8	41.6	54.6	0
505 F	97	48	—	31.2	37.7	61.1	0
516 M	287	48	—	31.2	25.1	45.5	0
517 M	206	47	33.8	18.2	28.6	45.5	0
653 M	170	48	36.4	26.0	28.6	52.0	++
655 F	128	48	37.7	18.2	37.7	50.7	0
656 M	145	48	27.3	31.2	29.9	57.2	0
657 F	143	48	42.9	24.7	33.8	46.8	+
658 F	162	48	45.5	23.4	36.4	46.8	+
659 M	159	48	33.8	14.3	39.4	48.1	0
660 F	144	48	33.8	15.6	—	41.6	0
661 M	175	48	33.8	—	—	—	0
663 M	124	48	31.2	23.4	37.7	54.6	0
683 M	67	48	39.0	19.5	—	40.3	0
(Mean) Average*			36.0 ± 1.32	24.1 ± 1.68	35.2 ± 1.24	50.3 ± 1.71	
Range			27.3 – 45.5	14.3 – 33.8	28.6 – 41.6	40.3 – 61.1	

*Animals 506 M and 507 M were not included in the computation.

Investigations and measurements were made of the dentin close to the formative end and in the middle third of the convex side of the upper incisor.

The following measurements (Fig. 1 and Tables I and II) were made in order to find the rate of dentin apposition: (1) the width of the predentin; (2) the distance of the experimental dentin line from the surface of the pulp; and (3) the distance of the calciotraumatic line from the surface of the pulp.

FINDINGS

Controls.—The control animals which had been starved for forty-eight hours showed no structural changes in the dentin of the incisors (Fig. 2). However,

the width of the predentin was significantly reduced (Table I). In the middle third of the incisor it averaged 13 microns, compared to a normal average of 16 microns.[3] This difference is probably a result of the starvation period of forty-eight hours.

Effects of Bilateral Nephrectomy.—All nephrectomized animals showed dentinal changes. These changes, with certain characteristic variations, repeated the pattern reported in other experiments affecting mineral metabolism. The pattern could be discerned most clearly in the middle third of the convex side of the upper incisor and gradually became less distinct toward the formative end of the tooth. The dentin appeared to be normal in the youngest part of the tooth.

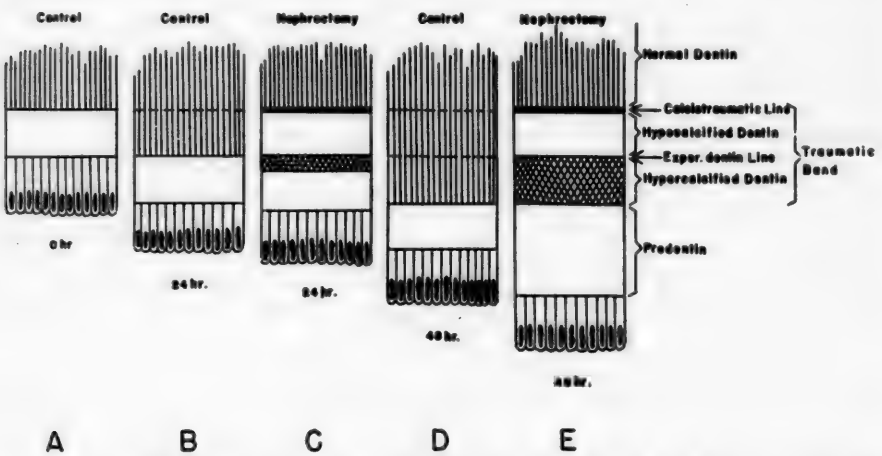

Fig. 1.—Diagram showing the calciotraumatic response of the dentin to bilateral nephrectomy. *A*. Control at beginning of experiment. Note normal width of predentin adjacent to normal, calcified dentin. *B*. Control twenty-four hours after beginning of experiment. Note newly formed layer of predentin. Predentin of previous day is calcified. *C*. Twenty-four hours after nephrectomy. Note calciotraumatic line, hypocalcified layer corresponding to predentin formed previous to start of experiment, hypercalcified layer, and predentin. Increment measured by distance of experimental dentin line from the pulpal surface is slightly greater than in control (*B*). *D*. Control 48 hours after experiment. Note additional layer of recently formed predentin. Predentin of *B* has become calcified. *E*, Forty-eight hours after nephrectomy. Note calciotraumatic response and increase in thickness of hypercalcified layer and predentin as compared with *C*. Increment measured by distance of experimental line to pulpal surface is significantly greater than in *D*. Compare with Figs. 3 and 4.

Photomicrographs of longitudinal sections of the upper incisor showing two types of dentin reaction to bilateral nephrectomy. Hematoxylin-eosin stain. Field is indicated in insert. Magnification ×850. *Od* = odontoblasts; *Pd* = predentin; *D* = normal, calcified dentin; *Her* = hypercalcified dentin; *Ho* = hypocalcified dentin; *EDL* = experimental dentin line; *CTL* = calciotraumatic line.

The traumatic band (Fig. 1) in the dentin was essentially the same as that described in detail in a previous report on the effect of injections of strontium.[1] The calciotraumatic line stained deeply with hematoxylin (Figs. 3 and 4). The hypocalcified layer was characterized mainly by its eosinophilia similar to that of predentin, but it also contained a number of globules stained with hematoxylin resembling globular predentin. This layer corresponds to the pre-experimental predentin. Its thickness (15 microns) can be considered normal. It decreased in width toward the formative end and finally disappeared at the point where the calciotraumatic line ended. The hypercalcified layer (Figs. 1, 3, and 4) was

characterized by an increased affinity for hematoxylin. It decreased in width toward the formative end. While the calciotraumatic line and the hypocalcified layer disappeared toward the formative end of the tooth, the hypercalcified layer, on the other hand, extended farther posteriorly in some teeth where it remained visible as an intensively stained line at the edge of the calcified dentin.

Fig. 2.

Fig. 3.

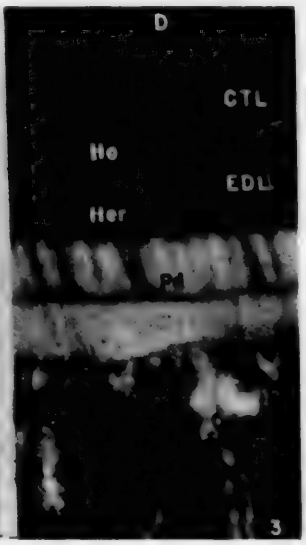

Fig. 4.

Fig. 2.—(Specimen 502) Control. Note homogeneous calcification of calcified dentin (*D*) and width of normal predentin (*Pd*).
Fig. 3.—(Specimen 653) Forty-eight hours after nephrectomy. Note calciotraumatic line, hypo- and hypercalcified layers, experimental dentin line and extremely wide predentin.
Fig. 4.—(Specimen 657) Forty-eight hours after nephrectomy. Calciotraumatic response differs from previous specimen by presence of additional line (*L*), which divides predentin into two zones.

The experimental dentin line marks the border between the pre-experimental and the experimental dentin. Measurements from this line to the surface of the pulp gave the amount of dentin and predentin formed during the experimental period of forty-eight hours. The average amount of experimental dentin formed in forty-eight hours was 35 microns (Table II). The width of the predentin of the control animal, that is, the increment produced in twenty-four hours, averaged 13 microns per twenty-four hours (Table I) and 26 microns in forty-eight hours in the middle third of the incisor. The rate of dentin formation was therefore greater in the nephrectomized animals.

The predentin of the nephrectomized animals was wider than that of the normal animals in both the middle and the formative third of the teeth (Tables I and II). A few minute calcospherites were found, especially in the formative third of the tooth. In four animals the predentin was divided into two layers by a distinct line (Fig. 4). The outer layer of the predentin appeared to be of a texture similar to that of the hypercalcified layer but was stained more lightly, while the inner layer appeared to be normal predentin.

Changes in the Pulp.—In three instances ectopic calcification of slight to moderate degree was observed in the mid-portion of the incisor. Fine granules, staining with hematoxylin, were dispersed in the intercellular substance and sometimes accumulated around the blood vessels.

DISCUSSION

General Pattern.—The dentin of the nephrectomized rats showed the same calciotraumatic response[1] as that described after the injection of the parathyroid hormone,[4, 5] calciferol,[4] fluorine,[6, 7] and strontium.[1]

Previous investigations[1, 7] have shown that the hypocalcified layer represents the pre-experimental predentin which remained uncalcified. This is confirmed by the present study in which the average width of the hypocalcified layer was found to be virtually the same as that of the predentin of normal animals (Figs. 1-4). Previous studies[1] also showed that the hypercalcified layer was the dentin formed immediately following the injury and was characterized by a reduction in the number of the fibrils and consequently by an excess of calcifiable cementing substance.

Different Reactivity of Incisal and Formative Parts of the Incisor.—The reaction was more severe in the middle third of the tooth than in the formative third. This difference was observed in most of the experimental procedures[7, 8, 9] studied, with the exception of strontium chloride injections where only a slight quantitative difference could be observed between the formative and the incisal parts of the incisors.[1]

An explanation for the more intensive reaction of the incisal parts may well be that the young odontoblasts at the formative end of the incisor are less susceptible to certain injuries than the odontoblasts that have already functioned for a longer period of time. Differences in the sensitivity of cells in different functional phases have also been observed in the ameloblasts.[10]

Rate of Incremental Growth.—Since the experimental dentin line represents the border between pre-experimental and experimental dentin, it can be used as a landmark for measuring the width of dentin formed after the experiment began. In nephrectomized animals this value at forty-eight hours averaged 35 microns (Table II). The incremental growth rate of the dentin of the control animals based on measurements of predentin width, was 26 microns per forty-eight hours. Thus nephrectomy had increased the incremental growth. We are unable to explain this surprising finding. Vitamin A deficiency[11] is the only other experimental procedure which is known to lead to accelerated rates of dentin appositions and then on the labial dentin only.

Calcification.—Under normal conditions the daily rate of calcification equals the rate of predentin formation. Under experimental conditions dentin formation and calcification may be altered independently. Therefore measurements of the width of the predentin under pathologic conditions give no indication of the daily rate of either formation or calcification. An increase in the width of the predentin, for instance, can be caused by an increase in apposition of dentin matrix or by a decrease in calcification. The rate of dentin formation can, however, be determined by reference to the position of the experimental dentin line. Such measurements permit also an estimate of the rate of calcification. After

nephrectomy the amount of dentin produced is greater than normal. However, only a relatively small portion (11 microns) is calcified.

Ectopic Calcification of the Pulp.—Calcification of the pulp was observed in three instances. McJunkin, Tweedy, and Menecky[12] under the same experimental conditions found, after forty-eight hours, necrosis of the myocardium. They assumed that the increase in inorganic blood phosphorus values was the cause of the damage to the tissues. This interpretation was supported by their findings that after bilateral nephrectomy and simultaneous injections of disodium hydrogen phosphate, necrosis and calcification of the myocardium could be found even after twenty-four hours. The findings of calcification in the pulp are in harmony with the findings of McJunkin, Tweedy, and Menecky.[12]

SUMMARY AND CONCLUSIONS

The effect of bilateral nephrectomy on the dentin and pulp was studied histologically in the upper incisors of twenty-two white rats. The findings were:

1. The dentin showed a characteristic traumatic band as the visible expression of the calciotraumatic response.

2. Using the experimental dentin line as a landmark indicating the border between pre-experimental and experimental dentin, measurements showed that the amount of dentin formed in the middle third of the incisor was increased.

3. Ectopic calcification of the pulp was found in a few instances.

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EXPERIMENTAL STUDIES IN CALCIFICATION

VIII. The Effect of Large Doses of Calciferol on the Dentin of the Incisor in Normal and Nephrectomized Rats

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THE development of the dentin proceeds in two well-defined steps of formation and of calcification. Previous experiments have shown that the formation of the organic ground substance and its mineralization may be influenced separately. It was shown that after nephrectomy the rate of formation of dentin was accelerated while the calcification was not disturbed.[1] It is also known that vitamin D primarily influences the calcification of hard tissues. Therefore, it seemed of interest to investigate the combined effect of nephrectomy and the injection of massive doses of calciferol.

MATERIAL AND METHODS

This study is based on the investigation of the upper incisor of three groups of Wistar albino rats. They were maintained on a diet of Fox Chow supplemented by meat scraps and cabbage leaf twice a week. All experimental and control animals were starved for the duration of the experiment (from 27 to 48 hours) but had free access to water.

Group A.—A group of nine animals was used as controls. Data on this group are found in Table I of a previous paper.[1]

Group B.—Nine animals were injected subcutaneously with calciferol in oil, in doses varying from 307,000 to 1,860,000 I.U. They were killed after 48 hours (Table I).

Group C.—Thirty-eight animals were bilaterally nephrectomized and were given simultaneously subcutaneous injections of calciferol. The doses were the same as for Group B. Five of the animals were sacrificed and examined after 27 to 30 hours and the rest after 46 to 52 hours (Tables II and III).

Examination of the Teeth.—The decalcified upper incisor teeth were cut in longitudinal sections and stained with hematoxylin and eosin. The following measurements were taken from the landmarks used in the preceding report[1]: (1) the width of the predentin, (2) the distance of the experimental dentin line from the surface of the pulp, and (3) the distance of the calcio-traumatic line from the surface of the pulp.

Serum Calcium Determinations were made in seven animals of Group B and twenty-four of Group C just before they were killed. The method has been described previously.[2]

FINDINGS

Group A. Controls

The dentin showed homogeneous calcification (Fig. 1, *A*). The width of the predentin averaged 13 microns. This is less than the normal 16 microns daily rate of dentin apposition.[3] The pulp was normal.

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TABLE I

EFFECT OF CALCIFEROL ON SERUM CALCIUM AND FORMATION OF DENTIN OF NORMAL RATS FORTY-EIGHT HOURS AFTER INJECTIONS
(GROUP B)

NUMBER AND SEX OF ANIMAL	WEIGHT IN GRAMS	DOSAGE OF CALCIFEROL*	SERUM Ca MG. PER 100 C.C.	WIDTH OF PREDENTIN: FORMATIVE THIRD IN μ	WIDTH OF PREDENTIN: MIDDLE THIRD IN μ	DISTANCE OF: EXPERIMENTAL DENTIN LINE FROM PULP IN μ	DISTANCE OF: CALCIOTRAUMATIC LINE FROM PULP IN μ
559 M	127	0.33	13.8	19.5	10.4	22.1	45.5
565 F	138	0.33	11.9	18.2	9.1	23.4	36.4
593 F	160	0.50	12.0	20.8	9.1	33.8	54.6
594 F	150	0.50	12.9	11.7	10.4	31.2	33.8
595 F	155	0.50	13.3	15.6	11.7	39.0	54.6
596 F	138	0.50	11.3	13.0	9.1	29.9	54.6
599 F	182	2 × 0.5	12.5	18.2	10.4	24.7	42.9
600 F	178	2 × 0.5		13.0	11.7	26.0	35.1
606 F	148	3 × 0.5		14.3	10.4	20.8	32.5
			Average (Mean ± standard error)	16.0 ± 1.02	10.2 ± .33	27.89 ± 2.26	43.3 ± 5.18
			Range	11.7 – 20.8	9.1 – 11.7	20.8 – 39.0	32.5 – 54.6

*1 c.c. contains 1,000,000 I. U. of calciferol.

TABLE II

EFFECT OF CALCIFEROL ON SERUM CALCIUM AND, FORMATION OF DENTIN OF NEPHRECTOMIZED RATS ONE DAY AFTER INJECTIONS
(GROUP C)

NUMBER AND SEX OF ANIMAL	WEIGHT IN GRAMS	DURATION OF EXPERIMENT IN HOURS	DOSAGE OF CALCIFEROL	WIDTH OF PREDENTIN AT: FORMATIVE THIRD IN μ	WIDTH OF PREDENTIN AT: MIDDLE THIRD IN μ	DISTANCE OF: EXPERIMENTAL DENTIN LINE FROM PULP IN μ	DISTANCE OF: CALCIOTRAUMATIC LINE FROM PULP IN μ	DEGREE OF ECTOPIC CALCIFICATION IN PULP
556 M	117	27	0.33	48.1	14.3	19.5	29.9	0
558 M	146	28	0.33	46.8	14.3	19.5	27.3	0
563 F	133	30	0.33		21.7	19.5	31.2	+
567 M	260	28	0.50	46.8	18.2	18.2	29.9	0
580 F	153	30	3 × 0.5	40.3	19.5	22.1	29.9	+
			Average (Mean ± standard error)	45.5 ± 1.52	17.6 ± 1.29	19.7 ± 1.79	29.6 ± 0.57	
			Range	40.3 – 48.1	14.3 – 21.7	18.2 – 22.1	27.3 – 31.2	

Group B. Effects of Injections of Calciferol

Dentin.—The reaction was most prominent in the dentin of the middle third of the labial side of the tooth (Fig. 2). The effect, in all cases, was similar to that described by Schour and Ham[4] and Schour, Tweedy, Chandler, and Engel.[5] It consisted of (a) the calciotraumatic line on the inner edge of the normal dentin; (b) a hypocalcified layer; and (c) more internally, a rather wide hypercalcified zone which was bordered on the pulpal side by predentin of normal appearance but of reduced width, compared to that of the control animals (Fig. 1, *B*).

The calciotraumatic line was characterized by an affinity to hematoxylin. The hypercalcified layer was basophilic and showed a characteristic sky-blue appearance. It was frequently divided into three zones, a middle lightly stained area bordered by two intensively stained areas.

The border between the hypocalcified and the hypercalcified layers, that is, the experimental dentin line, was the pulpal surface at the beginning of the experiment.[6] The distance of the experimental dentin line from the pulpal surface at the end of the experiment is a measure of the rate of dentin formation. This distance averaged 27 microns. The daily rate of dentin apposition was therefore 13 to 14 microns which is the same as in the control (Table I).

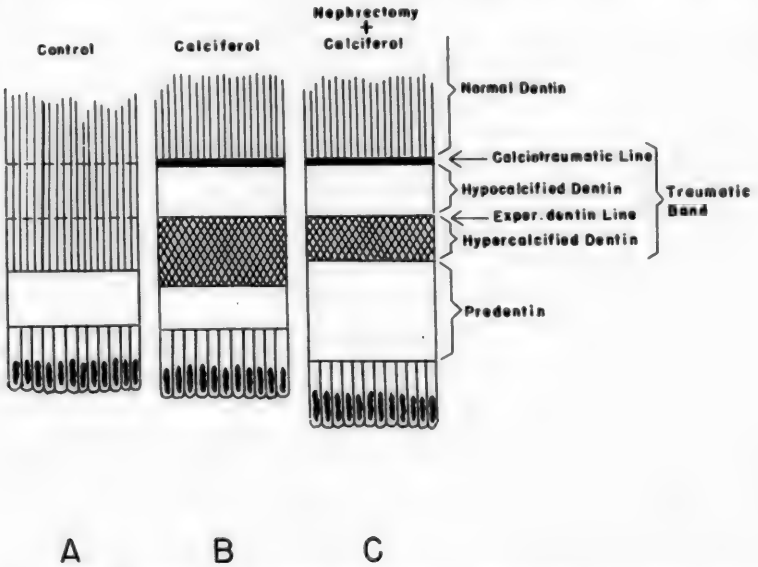

Fig. 1.—Diagram showing the calciotraumatic band after injection of calciferol in normal (*B*) and nephrectomized (*C*) rats.

A, Control animal. Note the normal width of predentin. The broken lines indicate the increments formed in 48 hours.

B, Forty-eight hours after injection of calciferol in normal rats. Note the calciotraumatic line, the hypocalcified and hypercalcified layers, and predentin. The distance of the experimental dentin line from the odontoblasts indicates the increments in 48 hours, which is virtually the same as in the control rats (*A*). The width of predentin is reduced, while the hypercalcified layer is increased in width.

C, Forty-eight hours after injection of calciferol in nephrectomized rats. Calciotraumatic response similar to that of *B*. Note that the incremental growth rate of dentin as indicated by the distance of the experimental dentin line from the pulpal surface, and the width of the predentin are increased.

Of the total dentin formed during the experiment, 17 microns were calcified while 10 microns remained as predentin. Thus the formation of dentin proceeded at the same rate as that of the control animals but the calcification was accelerated (Fig. 1, *B*).

Toward the formative end, the calciotraumatic response of the dentin became less distinct. The predentin in this part was narrower than that of the control animals.

Very little difference was found in the response of the dentin of the incisors to different doses of calciferol.

Pulp.—The pulp did not show any ectopic calcification although calcium values at death were higher than normal.

Group C. Effects of Calciferol Injections in Nephrectomized Rats

Dentin.—The reaction was most marked in the dentin of the middle third on the labial side of the incisor and essentially the same regardless of the different doses used. The pattern of the traumatic band, consisting of (a) calciotraumatic line, (b) hypocalcified layer, and (c) hypercalcified layer, was

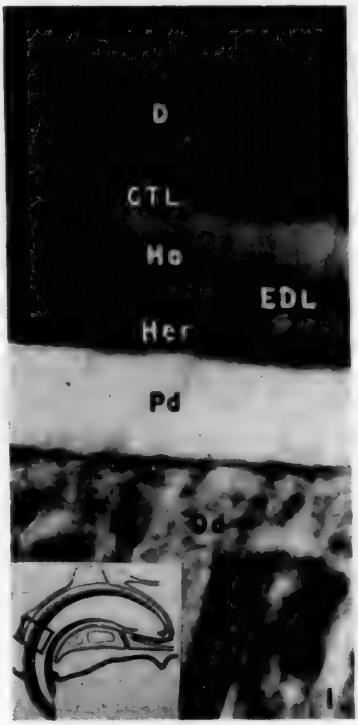

Fig. 2.—Photomicrograph of a longitudinal section of an upper incisor of Rat 569 which was nephrectomized and given injections of calciferol. This animal was allowed to live 48 hours after nephrectomy. The area is indicated in the insert. Mag. ×850.

Od = odontoblasts; *Pd* = predentin; *D* = normal, calcified dentin; *Her* = hypercalcified dentin; *Ho* = hypocalcified dentin; *EDL* = experimental dentin line; *CTL* = calciotraumatic line.

the same in all experimental animals (Fig. 1, *C*). As a result of the incremental growth of the dentin, the traumatic band was found farther away from the odontoblasts in the group that was allowed to survive 48 instead of 24 hours. The staining reaction of the different layers of the traumatic band was similar to that found after injection of calciferol in normal rats. The hypercalcified layer was frequently found to be divided into three zones, two peripheral more deeply staining borders and a central somewhat lighter zone (Fig. 2).

TABLE III

EFFECT OF CALCIFEROL ON SERUM CALCIUM, AND FORMATION OF DENTIN OF NEPHRECTOMIZED RATS TWO DAYS AFTER INJECTIONS (GROUP C)

NUMBER AND SEX OF ANIMAL	WEIGHT IN GRAMS	DURATION OF EXPERIMENT IN HOURS	DOSAGE OF CALCIFEROL	SERUM Ca MG. PER 100 C.C.	WIDTH OF PREDENTIN		DISTANCE FROM PULP IN μ		DEGREE OF ECTOPIC CALCIFICATION IN PULP
					FORMATIVE THIRD IN μ	MIDDLE THIRD IN μ	EXPERIMENTAL DENTIN LINE	CALCIOTRAUMATIC LINE	
557 M	144	48	0.33	9.8	36.4				0
560 M	197	52	0.40		31.2	11.7	29.9	37.7	0
561 M	185	51	0.40		31.2	11.7	29.9	46.8	+
562 M	167	46	0.33		33.8				0
564 F	129	48	0.33	13.9	40.3	26.0	32.5	44.2	+
568 M	220	47	0.40		33.8	22.1	32.5	41.6	0
569 M	234	50	0.50	12.7	41.6	16.9	26.0	39.0	+
570 M	245	49	0.50	10.7	46.8	22.1	46.8	61.1	0
571 M	232	49	0.40	10.6	44.2	19.5	29.9	46.8	0
572 M	231	47	0.40	12.7	41.6	18.2	33.8	44.2	0
573 M	283	48	2 × 0.5	9.8	32.5	23.4	39.0	52.0	+
574 M	260	48	2 × 0.5	9.8					0
575 M	252	48	2 × 0.5	9.5	32.5	15.6	36.4	49.4	++
576 M	231	48	2 × 0.5	9.7	31.2	24.7	42.9	58.5	+
577 M	239	48	2 × 0.5	10.2	33.8	23.4	40.3	55.9	+
579 F	148	48	3 × 0.5	15.0	36.4	22.1	27.3	41.6	0
581 F	149	48	3 × 0.5	19.8	45.5	29.9	40.3	46.8	0
582 F	154	48	3 × 0.5	17.1	37.7	22.1	26.0	36.4	0
583 F	144	48	3 × 0.5	15.8	40.3	28.6	39.0	49.4	+
584 F	142	48	3 × 0.5	17.3	45.5	31.2	35.1	50.7	+
585 F	143	48	3 × 0.5	15.7	36.4	35.1	44.2	55.9	0
586 F	152	48	3 × 0.5	16.0	35.1	36.4	37.7	50.7	0
587 F	129	48	3 × 0.5	16.0	48.1	18.2	39.0	49.4	±
601 M	162	48	3 × 0.5		37.7	22.1	31.2	41.6	±
603 M	156	48	3 × 0.5		40.3	27.3	31.2	44.2	±
604 M	153	48	3 × 0.5		33.8	22.1	28.6	40.3	+
607 F	174	48	3 × 0.5		31.2	20.8	24.7	36.4	+
608 F	150	48	3 × 0.5	13.2		20.8	29.9	41.6	0
609 F	137	48	3 × 0.5	16.9	53.3	28.6	42.9	55.9	0
610 F	138	48	3 × 0.5	16.1	39.0	16.9	32.5	24.7	±
611 F	170	48	3 × 0.5	17.5	42.9	32.5	44.2	50.7	+
612 F	188	48	3 × 0.5		49.4	16.9	27.3	36.4	±
613 F	289	48	3 × 0.5	12.6		28.6	37.7	50.7	0
				Average (mean ± standard error)	38.78 ± 1.09	23.1 ± 1.13	34.6 ± 1.12	46.0 ± 1.41	
				Range	31.2 – 49.4	11.7 – 36.4	24.7 – 46.8	24.7 – 61.1	

The incremental growth rate, measured by utilizing the experimental dentin line as a point of reference, was 20 microns in the first 24 hours and 14 microns in the second 24 hours. The increase of dentin apposition is equal to that seen after total nephrectomy. This was to be expected since calciferol did not influence the rate of dentin formation in normal rats. The hypercalcified layer averaged 11 microns in thickness (Table III) compared with 17 microns in the rats which had not been nephrectomized (Table I). While calciferol acted to increase the rate of calcification in non-nephrectomized rats, this effect seems to have been counteracted by the nephrectomy.

The serum calcium values at death after 48 hours varied between 9.5 and 19.8 mg. per 100 c.c. (Table III). Nephrectomized animals with high terminal serum calcium levels showed an additional finding in the incisor. A fine basophilic line was found approximately in the middle of the predentin layer.

The predentin at the formative end was much wider than in the animals given calciferol alone (Tables II and III) and was even wider than that found after nephrectomy.[1]

Pulp.—Ectopic calcification in the pulp was found in nineteen animals. All but two of them were animals killed after 48 hours (Tables II and III).

DISCUSSION

Calciotraumatic Response of the Dentin.—The results confirmed the histologic findings of Schour and Ham.[4] The timing of the reaction, as suggested by these workers and by Schour, Tweedy, Chandler, and Engel,[5] requires modification in view of the finding of Irving[7] that the junction of the hypo- and hypercalcified layers (the experimental dentin line) represents the border between the pre-experimental and experimental dentin. This interpretation, first arrived at after injections of fluorine, has been shown to be true also for the effect of strontium[6] and seems to apply generally to all calciotraumatic responses in the dentin. These experiments have shown that the hypocalcified layer represents the pre-experimental predentin while the hypercalcified layer is produced immediately following the start of the experiment. The width of dentin between the experimental dentin line and the pulpal surface is the amount of dentin formed after the beginning of the experiment.

Rate of Formation and Calcification of Dentin.—Comparison of the animals given injections of calciferol with those that were nephrectomized[1] showed a very interesting difference in addition to the similar calciotraumatic response. Calciferol did not alter the rate of predentin formation but accelerated significantly the rate of the calcification of the predentin. Nephrectomy, on the other hand, increased significantly the rate of formation of predentin while the rate of calcification remained normal.[1] The combination of the two experimental procedures furnished a peculiar result. Calciferol injected into nephrectomized rats did no longer exert its accelerating influence on the rate of calcification of the predentin. The acceleration of predentin formation, however, induced by the nephrectomy, continued and was not modified by the simultaneous injection of calciferol.

Ectopic Calcification of the Pulp.—In normal animals injected with calciferol no ectopic calcification of the pulp was found, though the calcium blood level in the animals was significantly increased (Table I). Nephrectomized animals showed foci of calcification in the pulp, though the calcium blood level in those animals remained normal.[1] From these findings the conclusion can be drawn that the calcification is primarily due to the toxic effect of phosphorus retention that creates focal necrosis. The injection of calciferol into nephrectomized rats did not significantly increase the incidence of pulpal calcification (Tables II and III). This finding strengthens the explanation of ectopic calcification as a consequence of focal necrosis.[8]

SUMMARY

The effect of large doses of calciferol on the dentin and pulp was studied in the upper incisors of nine normal and thirty-eight nephrectomized rats. The findings were:

1. The dentin showed the typical calciotraumatic response in both groups.
2. Calciferol in normal rats did not influence the rate of dentin formation but increased the rate of calcification.
3. Calciferol in nephrectomized rats lost its accelerating influence on the calcification of predentin.
4. The accelerating influence of nephrectomy on the rate of dentin formation was not influenced by simultaneous injections of calciferol.
5. Ectopic calcifications of the pulp were absent after administration of calciferol. Nephrectomized animals showed about the same incidence of ectopic calcification with or without injection of calciferol.

The authors wish to acknowledge their appreciation to Mrs. H. Kutuzov for technical assistance.

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EXPERIMENTAL STUDIES IN CALCIFICATION

IX. The Effect of Parathyroid Extract on the Dentin and Pulp of the Incisor in Normal and Nephrectomized Rats

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INTRODUCTION

THE effect of injection of parathyroid extract on the structure of the incisor of the normal rat was described in 1934 by Schour and Ham[1] and Schour, Tweedy, and McJunkin.[2] These investigators made no attempt to measure the incremental growth rate of dentin following the experimental procedure.

Irving, Weinmann, Schour, and Tweedy[3] reported that nephrectomy increased the amount of dentin formed in the forty-eight-hour experimental period. It is well known that parathyroid extract exerts a direct influence on the kidneys, reducing the reabsorption of phosphates.[4] In view of these facts it seemed interesting to combine the two experimental procedures and to examine the action of the parathyroid extract in nephrectomized animals. At the same time, quantitative studies on the influence of parathyroid extract on the rate of dentin apposition had to be undertaken in order to establish a base line for a detailed comparison of the changes induced by the injection of the parathyroid hormone in nephrectomized and nonnephrectomized rats.

METHODS AND MATERIAL

The study is based on the investigation of the upper incisor of three groups of Wistar albino rats. They were maintained on a diet of Fox Chow supplemented by meat scraps and cabbage leaf twice a week.

Group A.—The same animals as those in a previous report,[3] fed a basal diet, were used as controls.

Group B.—This group consisted of ten animals which were given 2 to 4 injections of parathyroid extract (50 Collip units per dose*) and sacrificed 24 to 48 hours later (Tables I and II).

Group C.—Twenty-seven animals were bilaterally nephrectomized and injected with parathyroid extract. Thirteen animals were sacrificed after 24 hours, fourteen after 48 hours (Table III).

The following measurements (Tables I to III) were made from the same landmarks as those used in the preceding reports:[3, 5] (1) The width of the predentin, (2) The distance of the experimental dentin line from the surface of the pulp, and (3) The distance of the calciotraumatic line from the surface of the pulp.

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*One collip unit is equivalent to 5 U.S.P. units of parathyroid extract.

TABLE I

EFFECT OF PARATHYROID EXTRACT ON DENTIN FORMATION AND ECTOPIC CALCIFICATION OF THE PULP TWENTY-FOUR HOURS AFTER INJECTION (GROUP B)

NUMBER AND SEX OF ANIMAL	WEIGHT IN GRAMS	DURATION OF THE EXPERIMENT IN HOURS	DOSAGE OF PARATHYROID EXTRACT IN COLLIP UNITS	PREDENTIN AT FORMATIVE END IN μ	DISTANCE FROM PULP		DEGREE OF ECTOPIC CALCIFICATION IN PULP
					EXPERIMENTAL DENTIN LINE IN μ	CALCIOTRAUMATIC LINE IN μ	
530 M	63	24	50 × 2	18.2	14.3	24.7	0
532 M	59	24	50 × 2	18.2	11.7	24.7	+
533 M	62	24	50 × 2	22.1	11.7	27.3	+ +
534 M	58	24	50 × 2	19.5	11.7	26.0	+ + + +
			Average (Mean ± standard error)	19.5 ± .56	12.35 ± .80	25.67 ± .54	
			Range	18.2 – 22.1	11.7 – 14.3	24.7 – 27.3	

TABLE II

EFFECT OF PARATHYROID EXTRACT ON DENTIN FORMATION AND ECTOPIC CALCIFICATION OF THE PULP FORTY-EIGHT HOURS AFTER INJECTION (GROUP B)

NUMBER AND SEX OF ANIMAL	WEIGHT IN GRAMS	DURATION OF THE EXPERIMENT IN HOURS	DOSAGE OF PARATHYROID EXTRACT IN COLLIP UNITS	PREDENTIN AT FORMATIVE END IN μ	DISTANCE FROM PULP		DEGREE OF ECTOPIC CALCIFICATION IN PULP
					EXPERIMENTAL DENTIN LINE IN μ	CALCIOTRAUMATIC LINE IN μ	
531 M	60	48	50 × 2	15.6	7.8	20.8	+ +
535 F	106	49	50 × 4	20.8	11.7	22.1	+ + + +
536 F	122	48	50 × 4	19.5	13.0	24.7	+ + +
537 F	105	48	50 × 4	16.9	10.4	26.0	+
538 F	112	48	50 × 4	18.2	18.2	26.0	+
539 F	94	48	50 × 4	14.3	15.6	26.0	+
			Average (Mean ± standard error)	17.55 ± .91	12.78 ± 1.38	24.26 ± .85	
			Range	14.3 – 20.8	7.8 – 18.2	20.8 – 2.6	

TABLE III

EFFECT OF INJECTIONS OF PARATHYROID EXTRACT ON FORMATION OF DENTIN AND ECTOPIC CALCIFICATION IN NEPHRECTOMIZED RATS AFTER TWO-DAY EXPERIMENTAL PERIOD (GROUP C)

NUMBER OF ANIMAL	WEIGHT IN GRAMS	EXPERIMENTAL DURATION IN HOURS	DOSE OF PARATHYROID EXTRACT IN COLLIP UNITS	WIDTH OF PREDENTIN		DISTANCE TO PULPAL SURFACE IN μ FROM		DEGREE OF ECTOPIC CALCIFICATION IN PULP
				FORMATIVE THIRD IN μ	MIDDLE THIRD IN μ	EXPERIMENTAL DENTIN LINE	CALCIOTRAUMATIC LINE	
509	263	47	50 × 4	36.4	26.8	28.6	40.3	0
510	152	49	50 × 4	42.9				+
514	269	49	50 × 4	44.2				0
515	148	49	50 × 4		18.2	24.7	37.7	0
547	112	49	50 × 4	44.2				0
548	111	48	50 × 4	27.3	13.0	26.0	37.7	0
549	100	48	50 × 4	37.7	16.9	29.9	39.0	0
550	122	48	50 × 3	16.9				0
566	127	48	50 × 4	31.2				0
597	135	48	50 × 3		16.9	27.3	37.7	0
598	137	48	50 × 3	16.9	14.3	31.2	44.2	0
614	107	48	50 × 4	29.9	19.5	31.2	45.5	+++
616	98	48	50 × 4	36.4	15.6	36.4	46.8	++
617	114	46	50 × 4	27.3				+
			Average (Mean ± standard error)	32.6 ± 2.62	17.6 ± 1.4	29.4 ± 1.22	41.1 ± 1.26	
			Range	16.9 – 44.2	13.0 – 26.8	24.7 – 36.4	37.7 – 46.8	

FINDINGS

Changes in the Dentin

All experimental animals showed the characteristic calciotraumatic response in the dentin of the incisors described previously for other experimental disturbances in mineral metabolism.[3, 6, 7] The reaction manifested itself as the traumatic band which consisted of three zones: (a) The calciotraumatic line, (b) The hypocalcified layer, and (c) The hypercalcified layer.

The dentin varied considerably from individual to individual in intensity, but not in essentials. The changes were most prominent in the middle third of the labial side of the incisor and became gradually indistinct toward the formative third, as in previous experiments.[3, 4] The modification of the calciotraumatic response characteristic for the two experiments (injection of parathyroid extract and the combination of bilateral nephrectomy and injection of parathyroid extract) will be described in detail.

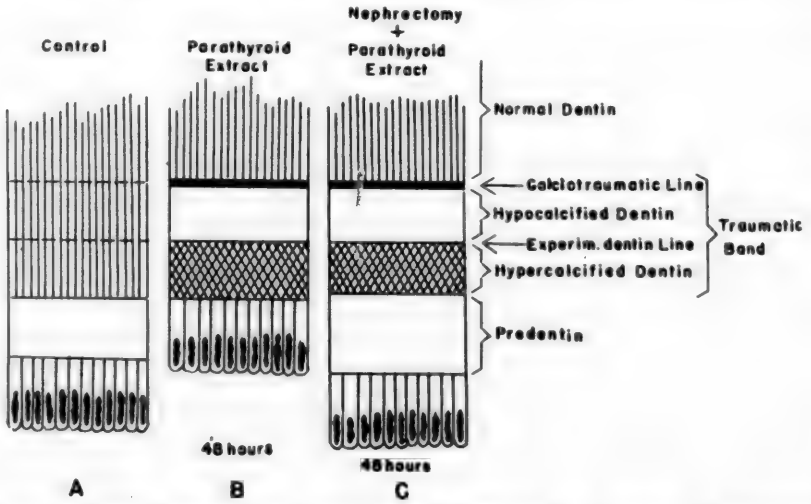

Fig. 1.—Diagram showing the calciotraumatic response after injection of parathyroid extract in normal (*B*) and nephrectomized (*C*) rats.

A.—Control animal (*A*). Note the normal width of predentin. The horizontal interrupted lines indicate the increments formed during the 48 hours of the experiment.

B.—Forty-eight hours after injection of parathyroid extract in normal rats. Note the calciotraumatic line, the hypocalcified and the hypercalcified layers, and absence of predentin. The distance of the experimental dentin line from the odontoblasts indicates the increment formed in 48 hours, which is significantly less than in the control rats (*A*).

C.—Forty-eight hours after injection of parathyroid extract in nephrectomized rats. Note the calciotraumatic line, the hypocalcified and hypercalcified layers. The distance of the experimental dentin line from the pulpal surface is slightly greater than that in the control rats (*A*) and so is the width of the predentin.

Group A. Controls (Fig. 1, *A*)

The control animals which had been starved for 48 hours showed no structural changes in the dentin. However, the width of the predentin averaged in the middle third of the incisor 13 microns, compared with the normal 16 microns daily rate of dentin apposition.[5] This difference is probably a result of the starvation period of 48 hours. The pulp was normal.

Group B. Effect of Parathyroid Extract in Normal Rats (Fig. 1, *B*)

The changes developed in the first 24 hours after the injection and were not modified during the second day (Tables I and II).

The Labial Dentin of the Middle Third of the Incisor.—The *calciotraumatic line* appeared as a fairly sharp and dark line.

The *hypocalcified layer* (the pre-experimental predentin) was structurally normal and characteristically stained a pale pink. The average width of the hypocalcified layer was found to be 13 microns, which corresponds to the width of the predentin in the middle third of the incisors of the control animals (Tables I and II).

The hypercalcified layer could be subdivided into two zones, an older and a younger zone. The older zone stained dark blue with hematoxylin, the younger zone light blue. The boundary between hypo- and hypercalcified layer (the experimental dentin line) was fairly sharp; that between the older and younger zones of the hypercalcified layer was often vague. A layer of predentin was absent in the middle third of the incisor. The hypercalcified layer was bordered by the odontoblasts.

Measurements of the distance of the experimental dentin line from the pulpal surface representing the amount of dentin formed following the beginning of the experiment showed that, in the first day of the experiment, the amount of dentin formed was virtually the same as the predentin of the control animals. The distance of the experimental dentin line from the pulpal surface did not increase during the second day of the experiment, proving that formation of dentin was arrested (Tables I and II).

Formative Third of the Labial Dentin.—In the formative third, the width of the predentin was reduced and averaged 18 μ, compared with the average width of 25 μ in the control animals. The structure of the predentin in this area appeared to be normal.

The transition between the conspicuous changes in the middle third of the tooth and the indistinct reaction of the formative end was gradual (Fig. 2).

The inner zone of the hypercalcified layer widened and changed its staining reaction from light blue to the characteristic pink staining of the predentin. The outer intensively blue-stained zone of the hypercalcified layer gradually decreased in thickness and became indiscernible as one progressed toward the formative end. Only in the areas where hypo- and hypercalcified layers were distinguishable could the calciotraumatic line be seen.

Pulp.—All animals but one showed varying degrees of ectopic calcification in the pulp (Tables I and II). The variation in degree was not found to be correlated to the serum-calcium level.

Two types of ectopic calcification were observed. Type A (Fig. 3) appeared as a diffuse sprinkling of the pulpal tissues with fine calcium granules, sometimes especially dense around the blood vessels. This type of calcium deposit was found mainly in the mid-portion of the incisor. Type B (Figs. 4 and 5) was always found close to the epithelial diaphragm (odontogenic epithelium). In this area the calcification was restricted to the intercellular substance of the

connective tissue. The fibroblasts seemed to be encapsulated and thus appeared almost like cartilage cells.

Some teeth showed both types of calcification; some only one. The second type was found in one animal after 24 hours. The others showed it only after 48 hours.

Group C. The Effect of Injections of Parathyroid Extract on Nephrectomized Animals (Fig. 1, *C*)

The *calciotraumatic line* was in most instances seen only in the middle third of the labial side of the incisor and became gradually indistinct toward the formative third. However, in three cases the calciotraumatic line was discernible also in the formative third. Since this line represents the border between calcified and uncalcified pre-experimental dentin, it is a significant landmark which presented in this case, for instance, not only an opportunity to analyze the changes in the middle third but also in the formative third of the incisor.

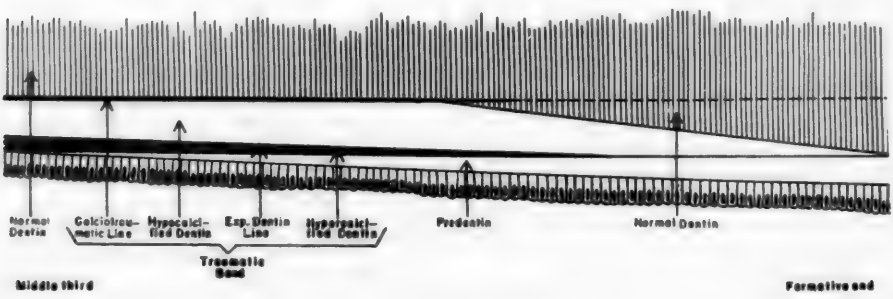

Fig. 2.—Diagram showing the difference of the calciotraumatic response between middle and formative thirds of the incisor after injection of parathyroid extract in normal rats. Note the fading of the calciotraumatic line and of the hypercalcified layer and the increasing width of the layer of predentin as one progresses from the middle to the formative third. At the same time the hypocalcified layer decreases in width and becomes gradually calcified.

Note the line in the predentin of the formative third. It represents the remnants and fusion of the hypercalcified layer and the experimental dentin line.

Photomicrographs of longitudinal sections of upper incisor of rats which were given injections of parathyroid extract. The areas are indicated in the insert. Specimens stained with hematoxylin-eosin.

P = pulp. *Od* = odontogenic epithelium. *D* = dentin. *A* = artificial space.

The *hypocalcified layer* was characterized by its affinity to eosin. The acidophilia was stronger in the middle third. Toward the formative third, the number of calcospherites increased and in the formative third this layer gradually approached normal calcification. This fact became evident in the few cases where the calciotraumatic line was discernible almost to the formative end. The average width of the hypocalcified layer (12 microns) was virtually the same as that of the predentin of the control animals (13 microns).

The *hypercalcified layer*, characterized by its affinity to hematoxylin, was split in most cases into three layers—peripheral, middle, and pulpal. The thickness of the three sub-layers differed. The peripheral and pulpal layers were intensively stained, the middle layer only lightly.

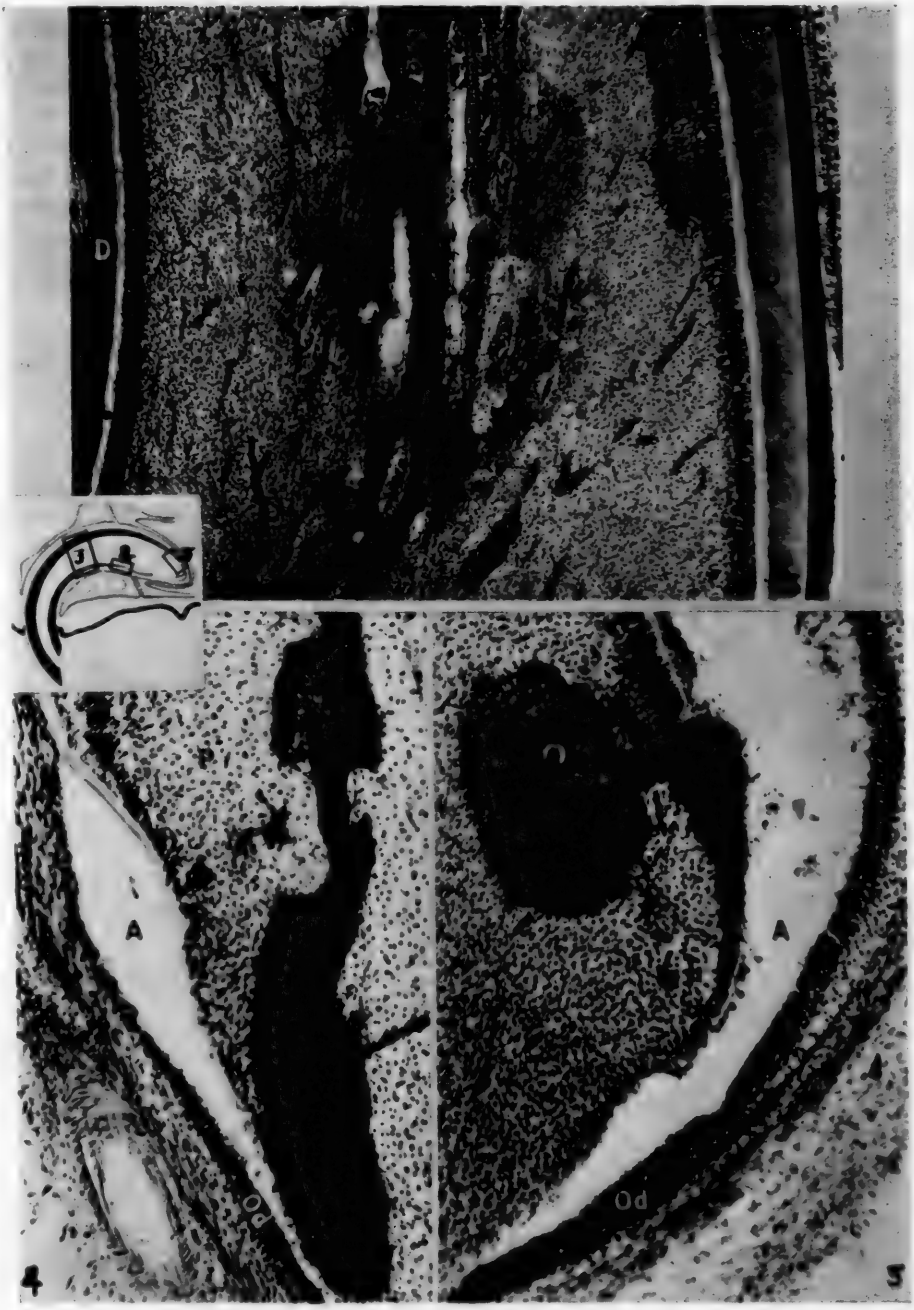

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 3.—Diffuse calcification in the center of the pulp and adjacent to convex side of the tooth (Type A).

Fig. 4.—Dense calcification at the pulpal surface of the odontogenic epithelium (*Od*) on the concave side of the tooth (Type B).

Fig. 5.—Dense calcification at the pulpal surface of the odontogenic epithelium (*Od*) on the convex side of the tooth (Type B).

The distance of the experimental dentin line (marking the border between the pre-experimental and experimental dentin) to the pulpal surface was, on an average, 29 microns (Table III). The calculated rate of dentin formation, if formation had progressed normally, was 26 microns for 48 hours (from the width of the predentin of the control teeth in the corresponding area). These figures show that the rate of dentin formation in nephrectomized animals that were given injections of parathyroid extract was increased, but not as much as nephrectomized rats without treatment with parathyroid extract[3] (35 microns). The *predentin* was wider than that of the control animals in the formative and in the middle third of the incisor.

Pulp.—In nine instances out of twenty-seven, ectopic calcification was found in the mid-portion of the incisor (Type A). In only one of these cases had calcification occurred close to the odontogenic epithelium (Type B).

DISCUSSION

The Calciotraumatic Response of the Dentin.—The changes in the dentin after injections of parathyroid extract confirm the findings of Schour and Ham[1] and Schour, Tweedy, and McJunkin.[2] The pattern of the calciotraumatic response was the same as those after injection of fluorine,[8, 9] calciferol,[1, 2] and strontium,[7] and following bilateral nephrectomy.[3] These experiments lead to the conclusion that the primary effect of an injury of short duration upon the formation and calcification of dentin is similar in pattern and is evidenced as a traumatic dentin band.

Rate of Dentin Formation.—In contrast to the similarity of the calciotraumatic response, the rate of dentin formation was affected differently following various experimental procedures. The rate of dentin formation was not affected by calciferol,[6] fluorine,[9] or strontium.[7] It was increased following nephrectomy[3] and decreased by injections of parathyroid extracts.

The inhibition of dentin formation is comparable to the primary effect of parathyroid extract on bone. Here the earliest sign is a disappearance of the osteoblasts.[10] That the arrest in dentin formation was not followed by resorption, which is so characteristic for the bone changes in hyperparathyroidism, may be explained by the fact that resorption does not constitute a factor in the normal biology of dentin.

McLean and Bloom[10] have shown degenerative changes of the osteocytes under the toxic effect of parathyroid extract. McJunkin, Tweedy, and Breuhaus,[12] after injection of parathyroid extract, found necrosis in the myocardium and the parenchyma of the kidney. The reduction of dentin formation after injections of parathyroid extract may be explained as a consequence of a toxic injury to the odontoblasts.

Ectopic Calcification in the Pulp.—Ectopic calcification in kidney and heart following injections of large doses of parathyroid hormone was first described by McJunkin, Tweedy, and Breuhaus.[12] They found that a necrosis of the tissue preceded the precipitation of lime salts and assumed that the hormone injured "parenchymal cells by disturbing the calcium components of the tissue fluids of the cells themselves."

Thoma[13] described in a case of generalized osteitis fibrosa disturbance of dentin formation and the occurrence of abundant interstitial deposits of calcium salts in the pulp.

In the present study almost all animals showed pulpal calcification after injection of parathyroid extract. Although no morphologic evidence of necrosis was observed, it can be assumed that a toxic injury of the cells had preceded the precipitation of lime salts. This belief is strengthened by the depressing effect of parathyroid extract on dentin formation, which can be explained by a toxic damage to the odontoblasts.

Summarizing the findings on pulpal calcification, after various experimental disturbances of mineral metabolism, it seems evident that the calcifications occur only if there is a primary injury in the tissues. After nephrectomy the degeneration is caused by the retention of inorganic phosphorus. The toxic effect of an excess of parathyroid extract is well established.[12] The toxic damages after nephrectomy[3] or injection of parathyroid extract and after the combination of both procedures prepare the basis for ectopic calcification which occurs even if the concentration of calcium in the blood remains normal.

SUMMARY AND CONCLUSIONS

The effect of large doses of parathyroid extract on the dentin and the pulp was studied in the upper incisors of ten normal and twenty-seven nephrectomized rats.

1. The dentin showed in both groups the calciotraumatic response.

2. Using the experimental dentin line as a landmark, the amount of dentin formed after the injection of parathyroid extract was measured. Parathyroid extract decreased dentin formation in normal animals while nephrectomy increased the rate of dentin formation. Injection of parathyroid extract in nephrectomized animals reduced the stimulating effect of nephrectomy on dentin formation.

3. The reduction of dentin formation after injections of parathyroid extract is probably caused by the toxic effect of an excess of the extract on the odontoblasts.

4. Ectopic calcification in the pulp was an almost regular occurrence after injection of parathyroid extract while the incidence and extent was lower in nephrectomized rats. The calcification occurred in two different types.

5. The ectopic calcifications are the consequence of focal tissue degeneration under the toxic effect of the parathyroid extract or the hyperphosphatemia after nephrectomy.

The authors acknowledge their appreciation to Mrs. H. Kutuzov for technical assistance.

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AN ASPECT OF DIFFUSION: ITS POSSIBLE ASSOCIATION WITH DENTAL CARIES

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PRELIMINARY CONSIDERATIONS

IN 1943 the writer began some experiments to determine the effects of corrosion by various acids upon extracted teeth. Close observation of teeth so treated showed a lack of corrosion of the enamel in (1) the region where the crown of the tooth touched the vessel containing the acid and (2) the deepest parts of fossae and sulci. This same result was noted in the contact area when two teeth touching each other were subjected to acid treatment.

Fig. 1 is a photograph of a tooth which was placed in a 5 per cent sulphuric acid solution and was then immediately brought in contact with another tooth. After two hours the teeth were removed from the solution, separated and inspected. The photographed tooth shows an uncorroded area around the actual point of contact that existed during the experiment.

The teeth in Fig. 2 were placed in a 20 per cent HCl solution and then immediately brought into contact with each other. After two hours the teeth were removed from the solution and examined. The enamel is practically untouched in the actual contact and surrounding areas (where some surface gloss is still evident) while in other regions there is heavy corrosion and in some portions complete loss of enamel. Similar observations were made when other inorganic and organic acids were used on contacting teeth and contacting spheres of marble, steel, and glass. This lack of corrosion in contact areas, according to Rashevsky,[8] is indicative of certain diffusion phenomena.

This phenomenon is not restricted to the movement or diffusion of acid ions into contact areas, but applies to other (and perhaps all) electrolytes and nonelectrolytes. This fact makes quite obvious the need for considering the laws of diffusion in studying the physicochemical aspects of dental decay.

Brown[1] describes the lines of diffusive flow around two "emitting" spheres. His concept may apply in part to this study; however, we believe certain mathematical principles more fully explain the phenomenon. Rashevsky[2] has presented a mathematical analysis of the phenomenon.

To date, experimentation justifies the assertion that in order to demonstrate the phenomenon, molecules or ions must be lost from a solution by reaction with, or adsorption by, the contacting solid bodies under consideration. The former method was used to obtain necessary data although later it became evident that the latter technic would be just as suitable.

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EXPERIMENTAL PROCEDURES

In order to simplify the measurement of distances and areas involved, steel balls were utilized in the following experiments. Ball bearings, which were almost perfect spheres with smooth surfaces, proved excellent for this purpose. Their diameters could be determined with a fair degree of accuracy and the surface areas calculated.

The reaction of corrosion of steel with aqueous HCl solutions in the concentrations used in the experiments involves the following reaction:

$$Fe + 2\ HCl \rightarrow FeCl_2 + H_2\uparrow$$

Kleinberg, one of the collaborators in this study, by using the method of Thompson[4] determined the amounts of dissolved iron obtained by exposing 1000 sq. mm. of steel ball surface to 500 c.c. of HCl solution for 10 minutes. His results are shown in Table I. Determinations of the H-ion concentrations of acid solutions were made with a Beckman (model G) glass electrode potentiometer. Instead of complicating the study by using buffered solutions, large volumes (20 liters) of solution were used in each of the following experiments to reduce to a minimum pH changes due to chemical reactions.

Fig. 1.

Fig. 2.

Fig. 1.—*A* indicates actual contact point. *B* and *C* are outer limits of uncorroded area. *D* is corroded enamel. *E* is root of tooth.

Fig. 2.—Teeth contacted each other at *A*. *B* and *C* mark outer limits of uncorroded zone. *D* shows heavily corroded enamel. *E* shows complete loss of enamel.

TABLE I

AMOUNT OF IRON LOST BY CORROSION OF 1000 MM.² OF STEEL BALL SURFACE EXPOSED TO 500 C.C. OF HCl FOR TEN MINUTES

EXPERIMENT	pH	MG. OF FE LOST PER 40 C.C.	AVERAGE
A_1	3.49	.020	.022
A_2	3.49	.024	
B_1	2.85	.066	.073
B_2	2.85	.080	
C_1	2.40	.200	.202
C_2	2.40	.204	
D_1	1.99	.286	.259
D_2	2.14	.232	

Other experiments were performed to determine the loss in weight of the steel balls resulting from exposing them to corrosion for varying lengths of

time at different acid concentrations. The results are given in Table II. Fig. 3 shows the reaction resulting in corrosion as proceeding at a constant rate from 0 to 100 or more hours as each pH level studied. However, Fig. 4 shows that the relative rates of corrosion increase as the H-ion concentration of the acid increases.

TABLE II

AVERAGE RATE OF CORROSION OF STEEL EXPOSED FOR 0 TO 100 HOURS TO VARIOUS CONCENTRATIONS OF HCl

pH	MG./MM.^{2}/SEC.
1.37	1.26×10^{-6}
2.12	3.02×10^{-7}
2.76	2.43×10^{-7}
3.54	3.63×10^{-8}
3.71	3.50×10^{-8}

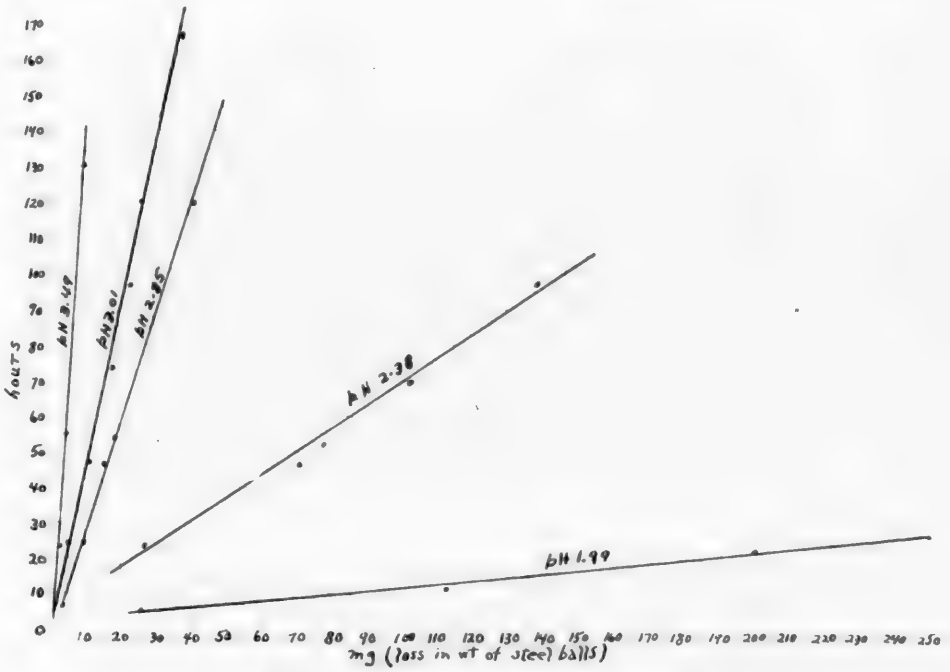


Fig. 3.—Rates of corrosion at various pH levels.

In the succeeding experiments, at least one-half of each ball was coated with ceresin wax to prevent great pH changes during the course of each experiment. The uncovered portion of a sphere was scrubbed with a soap solution, rinsed with distilled water, and brought into contact with another similarly treated sphere *after* it had been submerged in the solution in order to prevent the trapping of air in the contact areas. A specially constructed trough of glass tubing was used to hold the spheres in contact in the acid solution.

Fig. 5 is a diagram of the contact area of a steel ball which had been placed in contact with another steel ball and then immersed in an aqueous acid solution. It shows the general appearance of the uncorroded area about the contact point. In general the outermost rings (away from the contact point) are more heavily corroded than those nearer the central uncorroded area. In addition to the central area there are zones of no corrosion between the rings. Zone *A* is referred to as the "inner zone" and appears to be devoid of any appreciable corrosion. The largest or outermost ring is called the "outer zone." The diameter of the circles enclosing the zones was measured

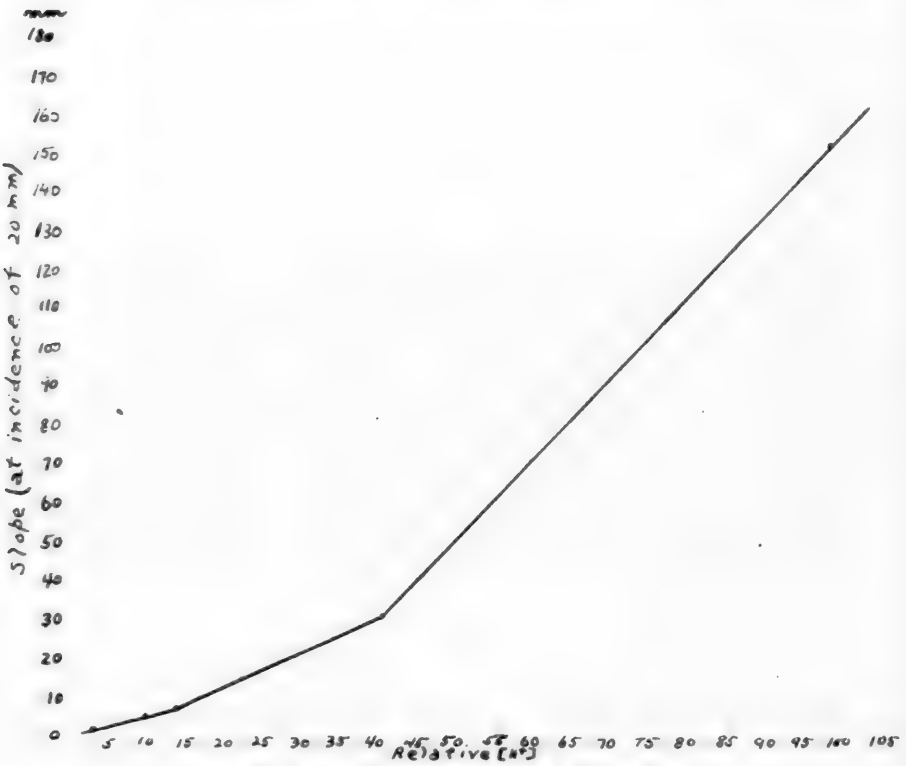


Fig. 4.—Relative rates of corrosion—0 to 100 hours.

with a caliper. The intermediate concentric rings or zones were not measured since their number and identity could not be readily established in all dissimilar experiments. The appearance of these concentric zones is quite similar to the Liesegang phenomenon described by D'arcy Thompson.[4]

Fig. 6 is a photograph of Liesegang rings produced by the author in the following manner: A gelatin solution containing a small amount of potassium bichromate was allowed to cool and set in a small watch glass. One drop of silver nitrate solution was placed in the center of the prepared gelatin mixture. In several hours concentric rings of precipitated silver bichromate appeared

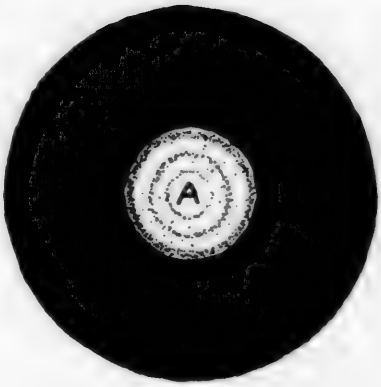


Fig. 5.

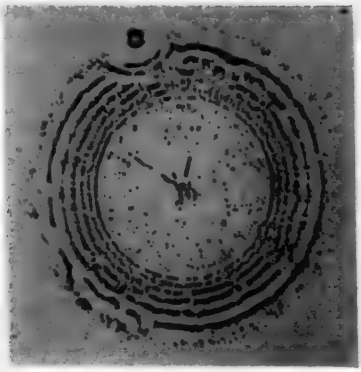


Fig. 6.

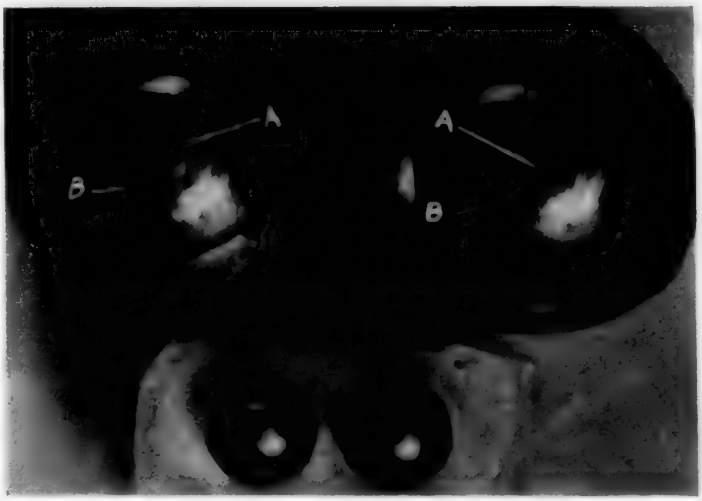


Fig. 7.

Fig. 8.

Fig. 5.—Contact point is in center of zone *A*. Visible are concentric rings of light corrosion with intermittent clear zones. Dark area of sphere is heavily corroded.

Fig. 6.—Liesegang phenomenon showing concentric rings of precipitated silver bichromate.

Fig. 7.—Large balls (diameter, 1 inch) were in contact with each other at *A*. *B* shows outermost ring. Intermediate rings are visible between *A* and *B*. The small balls show same phenomenon (diameter, ¾ inch).

Fig. 8.—*A* indicates progressive decrease in size of inner clear zone from 8 hours to 120 hours.

around the area where the silver nitrate was deposited. Liesegang rings are manifested throughout nature and are well known to be associated with certain diffusion phenomena.

Fig. 7 is a photograph of two pairs of balls which show the actual appearance of the zones and their size in relation to the size of the metal spheres on which they were formed. Both pairs of balls were treated in similar aqueous hydrochloric acid solutions (pH 3.38) for 48 hours.

Fig. 8 shows the relation between the length of time the balls were immersed in acid and the size of the inner (clear) zone. All three balls are 1 inch in diameter and show a decrease in the size of the inner zone with an increase in time of exposure to the acid solution. Ball 1 was exposed for 8 hours, Ball 2 for 48 hours, and Ball 3 for 120 hours.

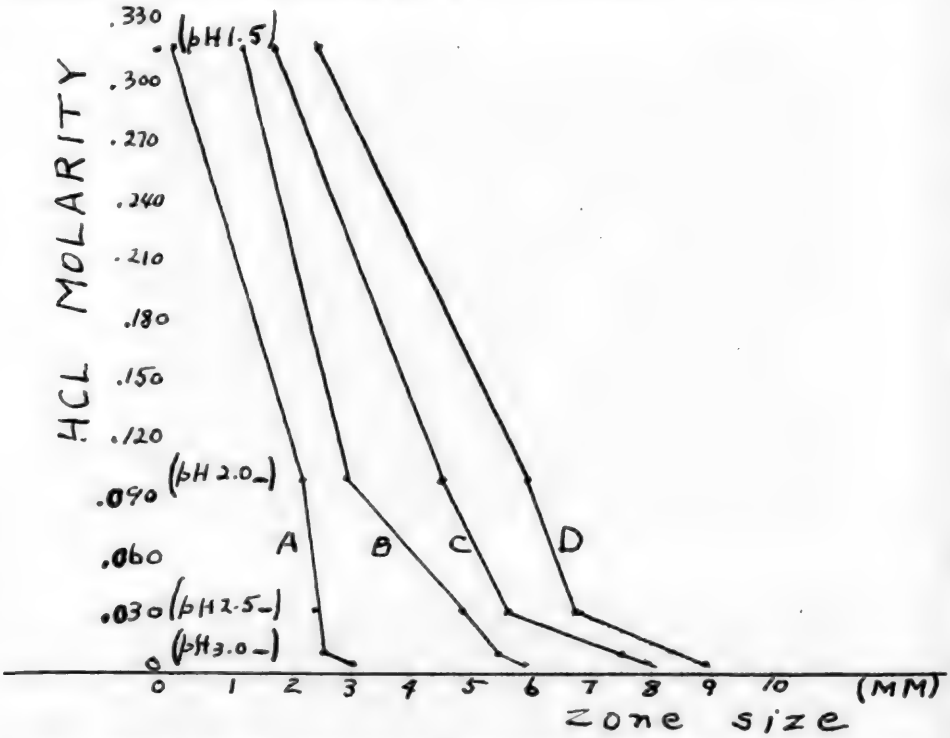


Fig. 9.—Size of outer zone related to acid concentration. Temperature 37° Centigrade. Time 48 hours.

Fig. 9 shows the relation between the pH of each aqueous solution and the size of the outer zones. Curve *A* represents measurements on balls 6.4 mm. in diameter; *B* represents balls 12.8 mm. in diameter; *C* represents 19.1 mm. in diameter; and *D* represents balls 25.5 mm. in diameter. As the pH decreases, the diameter of the outer zone also decreases regardless of the size of the steel balls. Fig. 10 shows a similar relationship between measurements of the inner zone and acid concentration. The irregularity of curve *A* is no doubt due to an experimental error.

Fig. 11 represents the relation between time of exposure and the size of the inner zone. The curves indicate like results for spheres of various sizes: the inner zone decreases in size with every increase in time of exposure to the acid solution.

Rashevsky[2] believed that the phenomenon here described is due to (a series of) chemical reactions limited primarily by disturbances in the physical processes of diffusion.

Some additional experiments were performed to reveal the influence of other physical variables on the behavior of diffusion in contact areas. Table

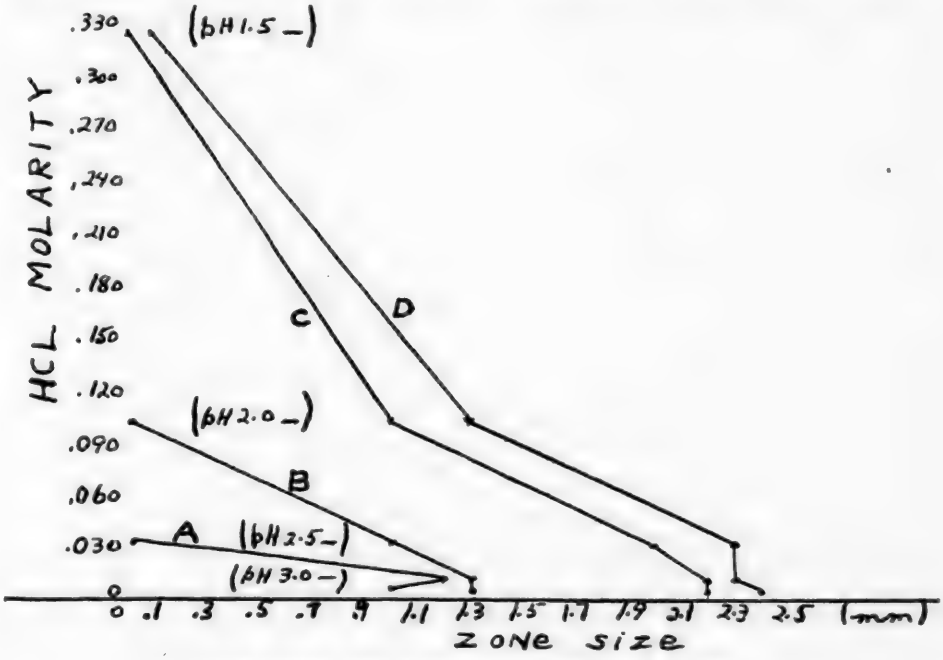


Fig. 10.—Size of inner zone related to acid concentration.

TABLE III
VISCOSITY EFFECTS ON ZONE FORMATION. DIAMETER OF BALLS 19.1 MM. TIME 48 HOURS. TEMPERATURE 37° CENTIGRADE

	STRAIGHT GLYCEROL + HCL		H_2O + HCL	
pH	INNER ZONE	OUTER ZONE	INNER ZONE	OUTER ZONE
1.0	3.5	5.5	0	0
1.5	3.7	7.1	0	1.5
2.5	0	0	2.0	5.5

III indicates that the viscosity of the solution can be a very important factor in zone formation and that the pH should not be considered alone in studying solutions dissimilar in other respects. Actually, corrosion in all areas of the spheres (away from the contact point regions) was reduced to a minimum in the more viscous glycerol solutions.

Table IV gives the results of separating the balls to varying degrees. It is obvious that disturbances of diffusion occur even with considerable separation of the balls. This sensitivity to changes in distance between the balls affords additional proof of the existence of important physical factors underlying diffusion phenomena.

TABLE IV

SEPARATION OF BALLS IN MM. AND SIZES OF ZONES. DIAMETER OF BALLS, 191 MM. TIME 48 HOURS. TEMPERATURE 37° CENTIGRADE

Separation	0	0.3	0.6	1.0
Outer zone	7.8	7.0	6.5	5.0
Inner zone	2.2	1.3	0	0

Table V shows the results of agitation with an electrical stirrer for 48 hours at a distance of 2 inches from the contact point. It is evident the zones are reduced in size but still visible after such treatment.

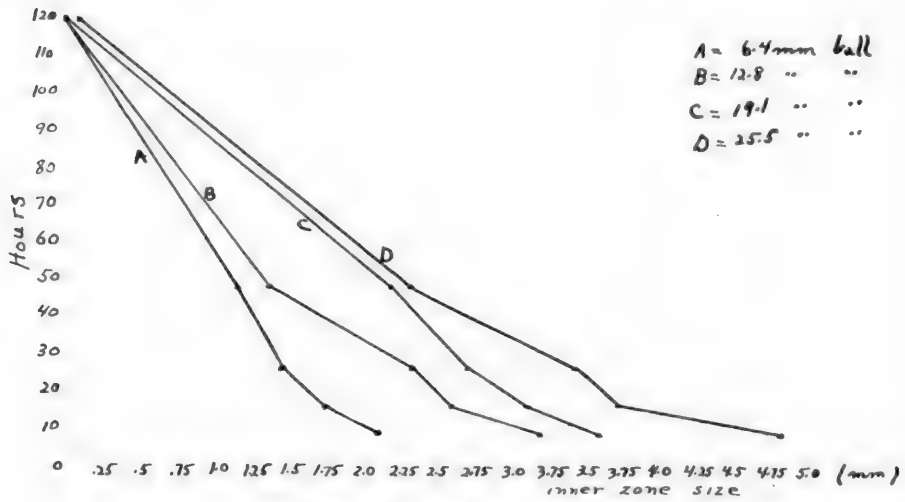


Fig. 11.—Size of inner zone related to time.

TABLE V

AGITATION OF SOLUTION WITH MECHANICAL STIRRER AND ZONE SIZES. DIAMETER OF BALLS, 25.5 MM. pH 3.5 TIME 48 HOURS. TEMPERATURE 37° CENTIGRADE

SPEED OF STIRRER	180 R.P.M.	300 R.P.M.
Outer zone	4.5	1.5
Inner zone	1.4	0

Table VI demonstrates that the outer limits of the outer zone are not affected appreciably by a moderate lowering of temperature. The inner (clear) zone, however, shows a decided change and increases in size as the temperature decreases.

TABLE VI

EFFECT OF TEMPERATURE ON ZONE SIZE. DIAMETER OF BALLS, 25.5 MM. pH 3.5. TIME 48 HOURS

	37° C.	2° C.
Outer zone	8.8	8.7
Inner zone	2.4	3.1

Table VII is self-explanatory and indicates that the introduction of various salts and other substances definitely affects the zone size. In all the $HCl+H_2O$ salt solutions the zones became smaller than those found in simple acid-water solutions. This indicates that the addition of salt facilitates the diffusion of HCl into the region of the contact point. These results coincide with observations made by McBain and Dawson[5] who noticed increase in diffusion rates of HCl in water solutions when small amounts of salt (KCl) was added. Their observations were made, however, on bulk solutions and "contact" areas were not considered. The last experiment in the column is exceptionally interesting. Under the conditions specified ($H_2O + HCl +$ egg albumin $+ KCl$) the greatest amount of corrosion occurred within the neighborhood of the contact point with relatively little corrosion of the balls in other regions. As shown in Fig. 12 *A* the entire neighborhood of the contact region is corroded more than the remaining surface of the ball.

TABLE VII

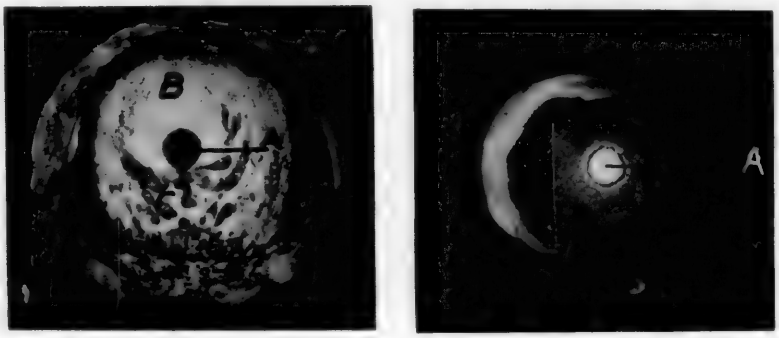
INTRODUCTION OF OTHER SUBSTANCES TO H_2O + HCL SOLUTION. DIAMETER OF BALLS, 19.1 MM. pH 3.5. TIME 48 HOURS. TEMPERATURE 37° CENTIGRADE

EXPERIMENT	SOLUTION	OUTER ZONE	INNER ZONE
1	H_2O + HCl	7.8	2.2
2	H_2O + HCl + .06M NaCl	7.1	1.9
3	H_2O + HCl + .06M KCl	7.0	1.0
4	H_2O + HCl + .06M KCNS	6.5	0.2
5	H_2O + HCl + Rb Cl .04M	7.0	2.0
6	H_2O + HCl + Ce Cl .04M	5.0	1.5
7	H_2O + HCl + gastric mucin (saturated)	6.0	4.0
8	H_2O + HCl + .01M sucrose	5.5	1.1
9	H_2O + HCl + ovalbumin (saturated)	3.3	1.0
10	H_2O + HCl + ovalbumin (saturated) + 10 Gm. KCl 3,000 c.c.	Heavy corrosion in contact area only (zone = 2.5 mm.)	

Protein (egg albumin) in solutions is a surface-active substance and probably accounts for the unusual behavior of diffusion in contact areas. A study of DuNoüy's[6] work is helpful in understanding why proteins in solution are adsorbed to surfaces and exhibit some well-defined characteristics. This phase of the study demands much more attention since teeth in life are covered with films and droplets of saliva containing protein.

The ball in Fig. 12 *B* was immersed (in contact with a similar ball) in an aqueous solution of $\frac{M}{1000}$ $CuSO_4$. Definite zone formation is evident. It shows that other electrolytes diffuse into contact areas similarly to acids.

In order to show that the phenomenon is not confined to the corrosion of steel by electrolytes, a dye was employed to show zone formation around contact points of specially treated glass spheres. Glass balls were coated with a 6 per cent solution of celloidin dissolved in equal parts of absolute alcohol and ether. After drying for 3 minutes, two such balls were placed in a .001 per cent solution of azure II and then immediately brought into contact with each other. Fig. 13 *A* shows the results after 3 hours and Fig. 13 *B* shows the unstained zone after 48 hours. The dark area is stained blue (color of the dye in water) and the clear area is unstained.



A. *B.*

Fig. 12*A*.—(Experiment 10 in Table VII.) *A* shows contact point with heavy corrosion in surrounding contact area. *B* shows light corrosion on remaining surface of sphere. Unexplained corroded pattern (*C*) below contact area prevalent in repeated experiments.

Fig. 12*B*.—*A* shows contact point in center of clear zone. $CuSo_4$ solution employed to corrode surface of ball instead of HCl.

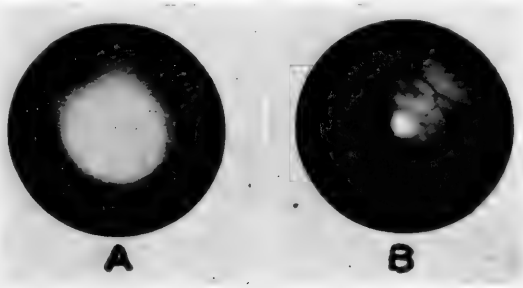


Fig. 13.—Actual contact points are in center of each white zone visible on both spheres. *A* shows zone size after 3 hours of treatment and *B* shows zone size after 48 hours.

In Vivo Demonstration of Retarded Diffusion into Contact Areas

To show that molecules diffuse with difficulty into contact areas in vivo, the author used his own oral cavity in performing the following experiment. A 1-½ inch piece of glass tubing, which was 5 mm. in diameter, was so arranged and fixed with modeling compound as to be in contact with the bucal surfaces of the lower right first and second premolar teeth when the mouth was closed. The exposed glass tubing was coated with 6 per cent celloidin, dried for 2 minutes, rinsed in distilled water, and then placed in position in the mouth.

Saliva was allowed to cover the tubing and form the usual fillet (droplet) in the contact regions formed by the glass cylinder and the two teeth. About 25 c.c. of .01 per cent methylene blue solution was taken and kept in the mouth for 5 minutes. After that period the saliva and methylene blue mixture was expelled and the glass tubing and compound were removed, rinsed in water, and examined. The celloidin-covered glass tubing was stained blue. In order to inspect the contact areas on the cylinder, the blue celloidin was scratched off of the tube on the side that approximated the cheek. Thus the unstained contact areas became visible when held before any light source.

Fig. 14 is a photograph of the appliance showing the results of the procedure. Several more trial experiments produced unstained contact areas in each instance, but their sizes varied each time. It was found impossible to control the agitation that resulted from involuntary muscular movements. In spite of such agitation unstained zones were produced, but naturally variations in their sizes occurred. Therefore, in the author's mouth, the methylene blue molecules did not diffuse (in 5 minutes) in any appreciable amount into the droplets of saliva present in the two pseudo-contact areas. Natural dental

Fig. 14.—Artificial appliance to form pseudo contacts with teeth. *A* is glass cylinder stained blue. *B* shows unstained contact areas. *C* is wax bite imprint holding cylinder in position in mouth.

contact areas, especially in the posterior regions, and deep occlusal grooves present much less divergence of the contacting tooth surfaces than did the curved surfaces involved in the artificial contact areas. It is permissible to assume that the methylene blue molecules would diffuse with greater difficulty in most natural dental "contact" areas in the author's own oral cavity (all other factors remaining constant) than in the case of the experimental contact regions.

THE ASSOCIATION OF DIFFUSION PROCESSES INTO CONTACT AREAS WITH DENTAL CARIES

Dental caries usually is initiated in (1) the neighborhood of contact points (areas), (2) the deepest parts of pits and grooves, and (3) the buccal and labial tooth surfaces near the gingival margin. All these areas have somewhat similar physical relationships. Two teeth in contact roughly resemble two spheres in contact. The deepest part of a pit or groove is comparable to the contact point of two spheres approximating each other with their curved surfaces diverging outward. The curved gingival areas of the buccal and

labial tooth surfaces and the curved gingival margin of gingival tissue roughly resemble two spheres in contact with each other. Denture clasps, loose crowns or fillings, etc., all create, between them and tooth structure, narrow spaces. It has been shown that molecules and ions in solutions diffuse with difficulty into such spaces.

If the concentration of natural protective salivary constituents in decreased in "contact" areas in one way or another, diffusion rates of these protective components from saliva elsewhere into contact regions must be equal to or exceed their rate of disappearance to maintain equilibrium between saliva and tooth structure. If the rates of loss of natural protective salivary components in contact areas exceeds the rates of their diffusion into these regions, dental decay may be expected to ensue.

A decrease in the concentration of protective salivary constituents in contact areas might result from (1) bacterial activity, (2) reactions with components of the enamel, and (3) physical diffusion into tooth structure.

Variations in the properties of the enamel in vivo may well account for the irregular distribution of areas of disintegration in one or both tooth surfaces involved in dental contact areas by the mechanism just described. Dobbs[7] and others have shown that all teeth as well as all regions of the same tooth (enamel) do not disintegrate at the same rate in vitro.

SUMMARY

1. Experimentally the ions or molecules of a solution diffuse with difficulty into the region around the contact points of spherical solid bodies immersed in liquid as predicted by the mathematical calculations of Rashevsky.

2. A similar delay in diffusion into contact regions has been suggested to occur in the human mouth.

The writer wishes to express his appreciation to Drs. N. Rashevsky, Jacob Kleinberg and T. B. Coolidge for their cooperation in securing the experimental data; to Dr. Rashevsky for his mathematical treatise of the phenomenon; to Dr. V. O. Hurme for assistance in the preparation of this paper; to Drs. C. E. Hatch, H. M. Marjerison, P. R. Howe, E. R. Serles, J. B. Blayney, and others, for support and encouragement; and to the University of Illinois Colleges of Dentistry and Pharmacy, Forsyth Dental Infirmary, and the Zoller Dental Clinic of the University of Chicago for laboratory facilities.

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ACID PRODUCTION IN SALIVA IN-VITRO EXPERIMENTS

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THIS paper deals with the formation of organic acids in the mouth and in in-vitro experiments as the result of sugar degradation and as indicated by pH changes.

Freshly secreted saliva gathered by canalization of a duct and collected under oil has a pH close to that of blood. Saliva secreted into the mouth and collected by spitting in a tube usually will have a pH of approximately 6.8 to 7. If this saliva is allowed to stand, particularly in a warm room, the pH will rise to between 7.2 and 7.8. If the saliva is filtered, the pH of the filtrate will rise as high as 8 or above. This change is due in part to the liberation of CO_2 and perhaps in some measure to the production of ammonia by the deaminization of proteinous materials.

It is an interesting fact that the production of acids in plaque surfaces within the mouth can not be duplicated in saliva collected from the mouth when the whole saliva is treated by in-vitro experiments. However, from in-vitro experiments much might be learned that may contribute to our understanding of acid production in the carious process.

Fig. 1 illustrates what happens to saliva collected by spitting when sucrose is added and incubated. This illustration shows only the extremes but the space between these extremes would be filled in if sufficient salivas were included. It is noted that some salivas drop in their pH value and others rise for the first hour. Possibly due to immediate buffer action, the greater portion of the pH drop comes in the latter part of the first hour and the first half of the second hour. These salivas which show pH drops or pH raises can not be correlated with lactobacilli counts.

It has been shown by Volker and Pinkerton[1] that sugar clearance of saliva in the mouth is largely within the first half hour. The work of Fosdick[2] and of Stephan,[3] and Fig. 2, will show that in the mouth the acid formed from additions of sugar occurs within a few minutes and at the end of thirty to forty-five minutes, the saliva returns to its normal pH. From this it would appear that the acid formed in the mouth as a result of carbohydrate degradation is the acid formed before the sugar has been cleared from the saliva. We shall therefore concentrate this discussion on that period immediately following the addition of sugar.

Fig. 2 shows the pH change in the mouth of an individual susceptible to caries after a 20 per cent sucrose rinse as recorded by an antimony electrode on the dorsal surface of the tongue. In the short period of eight minutes the pH has dropped to about 4.5 and returned nearly to normal within thirty

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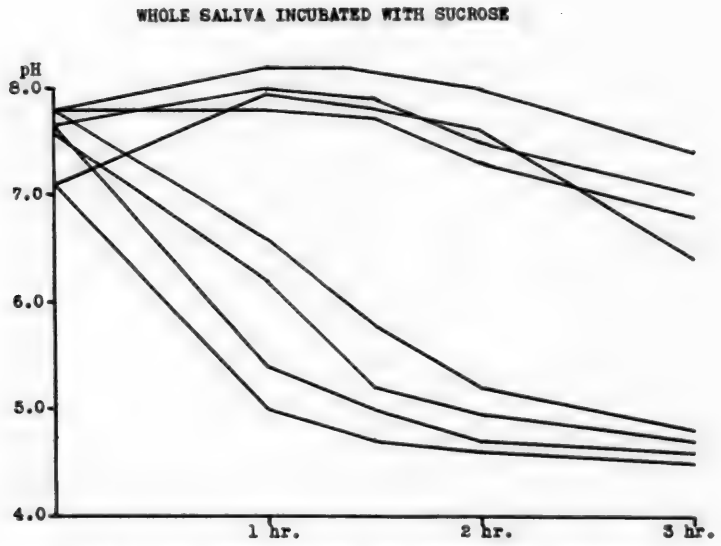

Fig. 1.—pH changes of saliva plus sucrose when incubated.

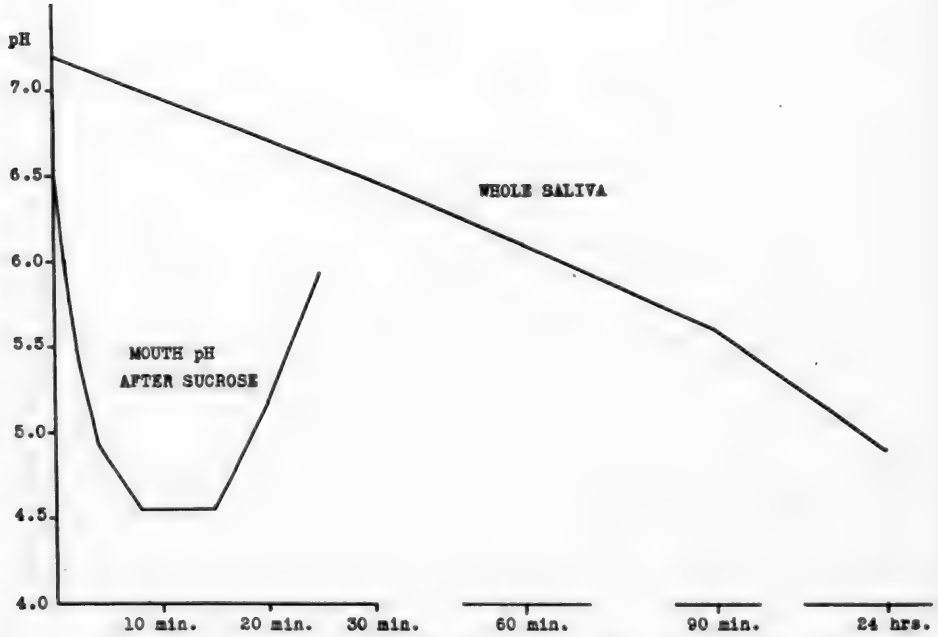

Fig. 2.—pH changes recorded on dorsum of tongue after a sucrose rinse and saliva from same individual plus sucrose when incubated.

minutes. This time relation corresponds to sugar clearance. The upper line shows the saliva from the same individual collected the same morning and to which additions of sucrose were made before incubating. The pH dropped slowly and only at the end of twenty-four hours did it attain a pH comparable to the mouth after four minutes. From this it is evident that some condition exists in the mouth that is not reproduced in a test tube. We are concerned with what that might be.

Tests with whole pooled saliva plus sucrose showed a similar drop in pH, that is, to about 6 in ninety minutes. If the same pooled saliva is filtered through cotton or paper, sucrose added and incubated, the pH rises for the first ninety minutes. If the saliva is Seitz filtered and additions are made to the filtrate of sucrose and 124 million lactobacilli per cubic centimeter, there is practically no pH change in the first two hours (Fig. 3.) This would indicate that there is something present in the whole saliva other than lactobacilli which accounted for the more rapid production of acid.

Fig. 4 shows the pH drop upon incubation after the addition of sucrose to pooled saliva which contained 38,800 lactobacilli per cubic centimeter. It also shows the effect of the addition of sucrose and 124 million lactobacilli to the filtrate from this same saliva. In spite of the fact that the filtrate contained 3000 times as many lactobacilli as the whole saliva and the same additional sucrose, the pH drop was only a fraction of that produced in whole saliva. Fig. 4 also shows the pH drop obtained when the sediment centrifuged from this same saliva, with additions of sucrose and saline, was incubated. Regardless of the fact that some, but not all, of the buffer has been lost by the substitution of saline for the filtrate of saliva, the rapidity of the acid formation approaches that which occurs in the mouth. The lactobacilli content but not the total bacterial count of this mixture is 1500 times less than that of the filtered saliva. The sucrose additions are the same. If this sediment is subjected to a temperature of 60° C. for twenty minutes (enough to destroy its enzymic content) before the addition of sucrose and lactobacilli, no acid is formed in the first ninety minutes. When 60 million lactobacilli are added to sucrose solution, more pH drop is observed than when 125 million are added to filtered saliva but not as much as by the sediment from centrifuged saliva. No buffer was added.

Fig. 5 shows the pH drops when the sediment obtained from pooled saliva is compared with the sediment obtained from broth cultures, both suspended in saline solutions with the same amount of sucrose added. In spite of the much smaller added bacterial content of sediment from saliva, the pH drop in ninety minutes was 2 pH units as compared with a pH drop of 1 pH unit from the sediment from broth cultures. This again indicates that the sediment is an important factor in acid formation.

Fig. 6 illustrates the result of treating pooled saliva from two groups of individuals in the same manner. The solid lines indicate incubated whole saliva plus sucrose, the lines composed of dashes indicate the same whole saliva plus

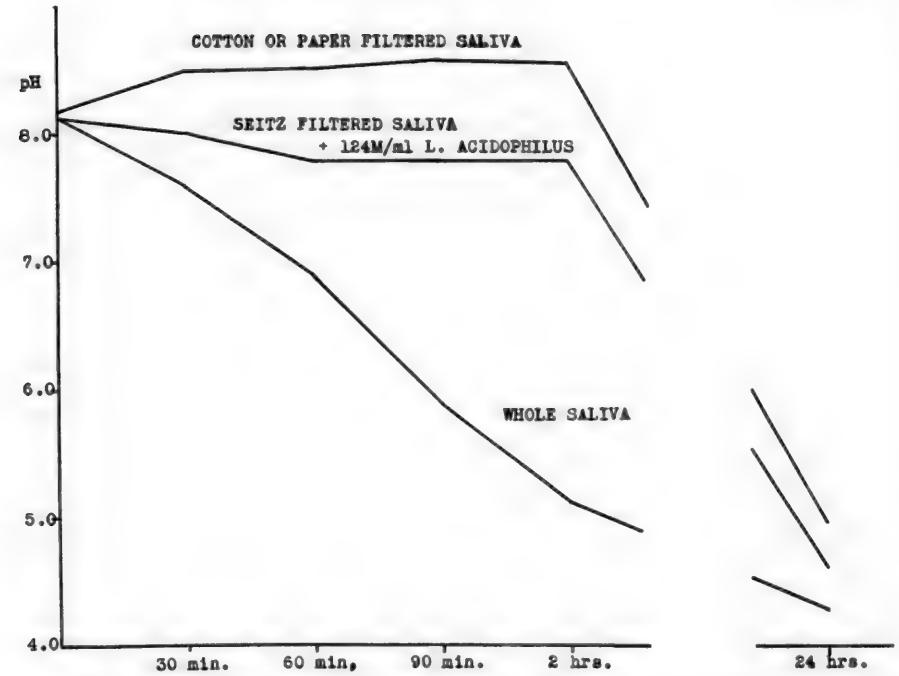

Fig. 3.—Difference in pH changes between whole pooled saliva and the same pooled saliva which has been filtered. Additions of lactobacilli to the filtered saliva do not make rapid formation of acid.

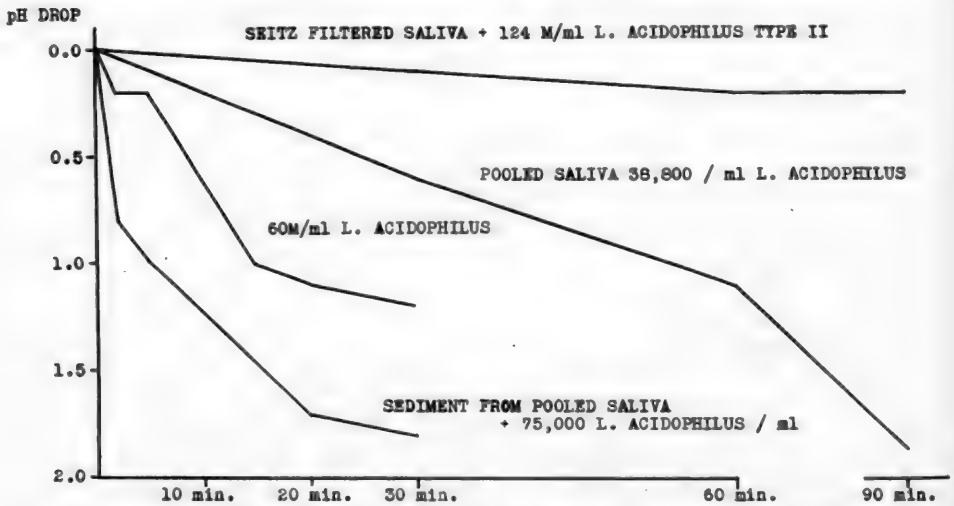

Fig. 4.—pH drop in pooled saliva containing 38,000 lactobacilli per cubic centimeter. Lack of acid formation of same saliva which has been filtered but to which large numbers of lactobacilli have been added. Rapid pH drop when the sediment from this filtered saliva is incubated.

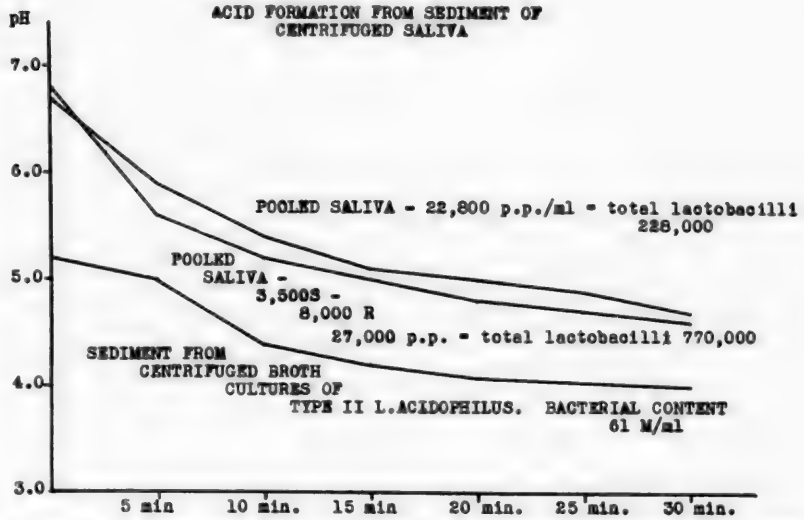

Fig. 5.—pH drop when sediment obtained from pooled saliva is compared with sediment obtained from broth cultures.

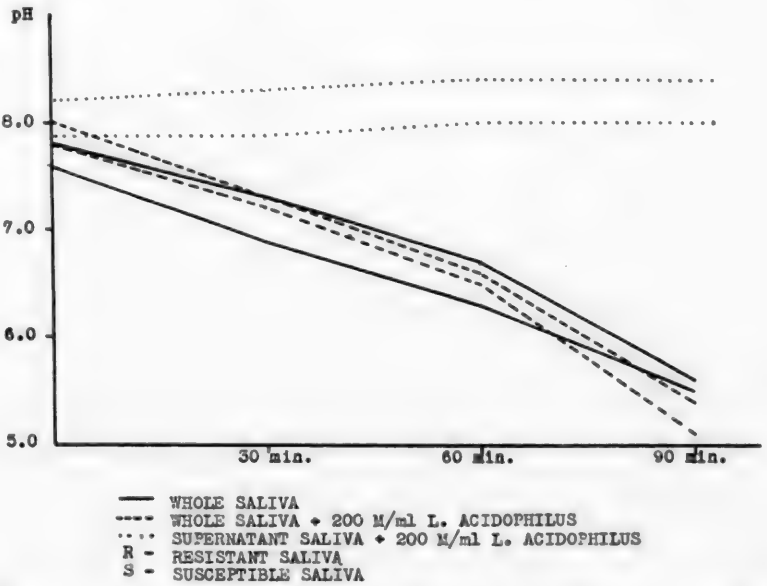

Fig. 6.—Pooled saliva from two groups of individuals when (a) sucrose has been added: (b) sucrose plus lactobacilli were added, and (c) supernatant fluid plus sucrose plus lactobacilli.

the same amount of sucrose plus 200 million lactobacilli (strain 4646) per cubic centimeter. It will be observed that the pH drops are essentially the same and the added 200 million lactobacilli per cc. make little or no different in the rate of acid formation. Similar tests on individual saliva conducted with a strain of lactobacilli isolated from the same individual produced comparable results. The lines composed of dots give the results on the supernatant fluids from the same saliva after centrifugation. This portion of the saliva also has the same amount of sucrose and 200 million lactobacilli per cc. added. No acid is formed in the first ninety minutes and the pH is slightly raised. In this test 8 mg. of sucrose was added per cc. of saliva. Other tests run in a similar manner with 200 million added lactobacilli per cc. indicate that the amount of acid formed is the same for the first ninety minutes regardless of whether 2, 8, 20, or 100 mg. per cc. of sucrose were added. At the end of twenty-four hours they show some difference but such difference is not evident in the first ninety minutes.

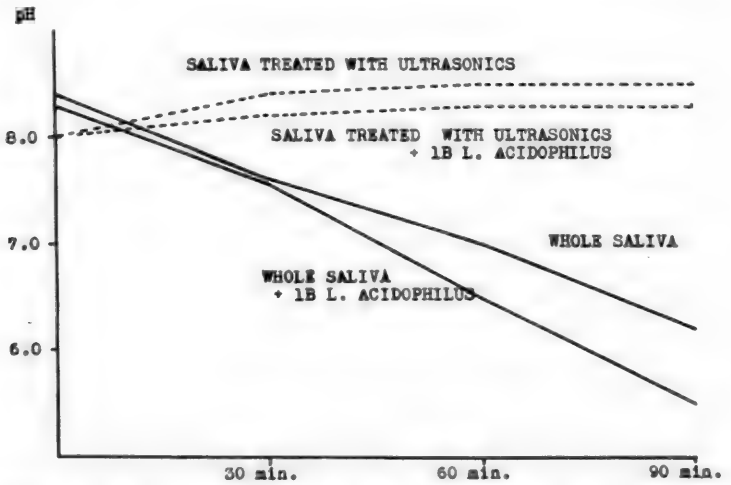

Fig. 7.—Pooled saliva incubated with and without additions of lactobacilli. The same saliva treated for ninety minutes with ultrasonics both with and without additions of lactobacilli.

In Fig. 7 the results of a similar test on pooled saliva is shown but with the addition of a billion lactobacilli per cubic centimeter. The additional organisms produced only a slightly greater drop in pH than that produced with 200 million organisms. In an attempt to determine what portion of the acid formed was produced by other organisms, the pooled saliva of susceptibles was subjected to ultrasonics for varying lengths of time. This figure shows the effect of ninety minutes exposure to ultrasonics. Such exposure to ultrasonics destroys most but not all of the organisms present. Cultures from this material after treatment shows the destruction of organisms to be fairly uniform and to consist of approximately 99 per cent reduction in total numbers. This treated saliva with sucrose added produces no acid in ninety minutes upon incubation. When

the same material was treated in the same manner, but with the addition of one billion lactobacilli per cc., no acid was formed but the raise in pH was slightly less.

CONCLUSION

From this evidence it is difficult to draw a definite conclusion but the suggestions are strong that factors other than the number of lactobacilli present play an important factor in acid production.

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THE EVANSTON DENTAL CARIES STUDY

III. A Preliminary Study of the Distribution of Fluorine* in Communal Water Supplies in the United States

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GENERAL interest and enthusiasm concerning the fluorine content of water, natural and artificial, has been increasing markedly since 1938. Scientific interest, especially in chemistry and dental medicine, stems from a desire to develop a method for the inhibition of dental caries. To the best of our knowledge, the role which fluorine plays in this regard is not understood completely.

The questionnaires used in the Evanston Dental Caries Study occasionally revealed that the children examined formerly have lived in areas in which fluorine is a natural constituent of the communal water. This paper was initiated and prepared to show: (1) the approximate location of areas where fluorine is prevalent in communal water supplies, and in what strengths, and (2) the number of people in the United States who are drinking, or have access to, a communal water supply in which fluorine is a natural constituent.

We believe it is important to know the number of people in the United States who are exposed to the various amounts of fluorine in the communal water supply. We also believe it is important to know the areas in which fluorine normally is found. Since it will take five to ten years to determine the trends in those areas in which fluorine is added artificially to the communal water, we proposed to determine the number of people living in the United States who daily are exposed to fluorinated water. We must realize that there are other sources of fluorides besides water supply. From recent studies carried on in our chemical laboratory,[1] we have evidence that many foods contain various amounts of fluorine. Also topical applications of sodium fluoride to tooth surfaces is receiving considerable attention, to say nothing of the practice of the laity to take tablets prepared by manufacturing pharmacists containing a fluoride compound.

REVIEW OF LITERATURE

The literature on this subject of fluorine in U.S. communal water supplies is extremely limited. The only reference, to our knowledge, is Van Burkalow's report entitled "Fluorine in United States Water Supplies."[2] By the ample

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*Fluorine when expressed as parts per million refers to the fluorine ion. Fluoride as used in this paper refers to the combined form, probably calcium fluoride or sodium fluoride.

use of maps, she has shown the distribution of fluoride-bearing waters in the various states of the Union, but has made no attempt to tabulate the number of endemic areas in each state or to list the total number of people who use such fluorinated water for drinking and cooking purposes.

For the material on which this study is based we wrote to the Chief Sanitary Engineers[3] of each State Health Department and the District of Columbia asking for the fluorine concentration of the communal water supply of each city, town, and village in their respective states. The sanitary engineers were most cooperative in supplying us with the desired information or referred us to proper sources. The data received from each state were segregated into four groups: (1) those listing fluorine content of communal water supplies of cities, towns, and villages, (2) those now conducting fluorine determinations on communal water supplies, (3) those showing all water supplies to be free from fluorine, and (4) those showing fluorine content of rural areas which were not considered in our tabulations (Table I).

TABLE I

SIXTEEN STATES AND DISTRICT OF COLUMBIA FURNISHING INFORMATION RELATIVE TO FLUORINE BUT NOT CONSIDERED IN TABULATION

WATER NOW BEING TESTED	NO FLUORINE DETERMINATIONS	MATERIAL BEING COMPILED	FLUORINE LESS THAN 0.5 PPM	INFORMATION NOT APPLICABLE TO STUDY*
Iowa	Delaware	Arizona	Pennsylvania	Alabama
New York	Kentucky		District of Columbia	Oklahoma
Montana	South Carolina		New Hampshire	North Carolina
West Virginia			Massachusetts	Oregon
			Maine	

*Rural areas: material on spot maps indicating no specific communal water supply.

Whenever a municipality has indicated that it obtains water from more than one source, we have assumed that all of these waters were distributed through a common distribution system. In some instances reporting communities have indicated that the fluorine content of the water, when drawn at its source, differed from the fluorine content of the same water when it was collected at some point in the distribution system. For the purpose of keeping uniformity, we have used only the figure indicating the fluorine content of the water as it was collected after passing through the distribution system. Some states have run fluorine tests annually since 1937, other states were active from 1937 to 1942. In the last five years, still other states have become interested and are now actively carrying on fluorine analyses (Table I). For the purposes of this paper all population figures are those derived from the 16th Census of the United States.[4] However, material recently released from the Department of the Census in Washington, D. C., suggests an over-all increase since 1940 of 10 per cent. Figures from the Bureau of Vital Statistics of Illinois suggest 4.09 per cent increase in population in the State of Illinois.[5]

TABLE II

NUMBER OF PEOPLE IN THIRTY-TWO STATES WITH 6299 COMMUNITIES LISTING FLUORINE IN STRENGTHS RANGING FROM 0.0 TO 5.1 PPM OR HIGHER

STATES	COM-MUNITIES	POP. OF STATE	FLUORINE CONCENTRATIONS (PPM)						
			0.0 TO 0.5	0.6 TO 0.99	1.0 TO 1.5	1.6 TO 2.0	2.1 TO 3.0	3.1 TO 5.0	5.1 AND HIGHER
Illinois	639	7,897,241	5,565,785	151,295	148,037	146,587	13,942	4,138	483
Texas	547	6,414,824	740,013	706,928	303,442	147,004	429,045	65,905	24,631
Ohio	438	6,907,612	3,664,459	103,348	82,652	25,995	32,501		
Minnesota	423	2,792,380	763,902	17,164	2,380	584			
Kansas	378	1,801,028	873,087	75,650	23,639	4,999		2,354	
Georgia	351	3,123,723	1,051,820	16,882					
Florida	317	1,897,414	816,480	210,790	35,330	7,491	837		
Wisconsin	283	3,137,587	1,634,826	48,069	4,050	22,479	52,608		
New Jersey	268	4,160,165	2,299,355	6,818	16,718	2,027			
Utah	251	550,316	387,156	5,985	1,693			290	
Indiana	248	3,427,796	621,663	126,682	50,074	12,626		1,448	
Massachusetts	238	4,316,721	4,019,838						
Missouri	220	3,784,664	610,140	43,111	35,669	3,442	2,414	3,749	
South Dakota	204	642,961	179,557	43,156	11,692	2,081	24,357	10,703	2,751
Tennessee	190	2,915,841	1,130,149	4,383		2,526			
New Mexico	159	531,818	110,337	49,206	33,690	18,598	3,912	415	
Nebraska	146	315,834	525,329	35,218	33,140	920			
Michigan	141	5,256,106	2,284,390	25,738	7,140	4,316			
Idaho	136	524,873	201,231	16,881	16,550			356	
Maryland	133	1,821,844	100,820	5,759	11,674	130	1,104	3,908	
Arkansas	94	1,949,387	281,271	12,342			5,349		
Mississippi	93	2,183,796	511,789						
Louisiana	80	2,363,880	266,486	37,769	65,967	502,630	1,615		7,872
North Dakota	71	641,935	180,553	21,672	9,272	3,803	3,102	22,544	261
Colorado	52	1,123,296	226,549	20,759	337,491	968	43,829	4,289	
Rhode Island	46	713,346	471,984	1,800	400				
Virginia	44	2,677,773	229,496	37,367		8.095	300	1,549	4,153
California	43	6,907,387	1,600,145	13,307	40,092	3,170	10,017	3,218	
Nevada	19	110,247	39,983		649	200	1,245	200	
Connecticut	16	1,709,242	514,716						
Wyoming	9	250,742	12,477	25,231	22,474			2,177	
Washington	24	1,736,191	156,544						
Totals	6299		32,072,330	1,863,310	1,293,915	920,671	626,177	127,243	40,151

TABLE III

INDICATES NUMBER OF COMMUNITIES IN EACH STATE WHICH CONSUMES WATER CONTAINING FLUORINE IN CONCENTRATIONS FROM 0.0 TO 5.1 PPM AND HIGHER

STATES*	COMMUNAL WATERS ANALYSED	FLUORINE CONCENTRATIONS (PPM)						
		0.0 TO 0.5	0.6 TO 0.99	1.0 TO 1.5	1.6 TO 2.0	2.1 TO 3.0	3.1 TO 5.0	5.1 AND HIGHER
Illinois	639	480	77	48	19	10	4	1
Texas	547	219	99	125	35	41	19	9
Ohio	438	319	41	49	22	7		
Minnesota	423	411	9	2	1			
Kansas	378	307	40	22	7		2	
Georgia	351	342	9					
Florida	317	271	24	18	4			
Wisconsin	283	255	17	4	5	2		
New Jersey	268	259	2	6	1			
Utah	251	225	18	6	1		1	
Indiana	248	174	42	25	6		1	
Massachusetts	238	238						
Missouri	220	190	12	12	2	2	2	
South Dakota	204	84	32	23	5	37	18	5
Tennessee	190	185	4		1			
New Mexico	159	52	41	44	10	9	3	
Nebraska	146	115	14	16	1			
Michigan	141	117	15	7	2			
Idaho	136	119	10	5		1	1	
Maryland	133	117	9	3	1	2	1	
Arkansas	94	85	4	3		2		
Mississippi	93	70	9	11	1	1		1
Louisiana	80	47	7	13	7	2	2	2
North Dakota	71	31	12	9	4	2	12	1
Colorado	52	29	11	6	3	1	2	
Rhode Island	46	42	2	2				
Virginia	42	29	1		4	2	3	3
California	43	20	6	2	6		8	1
Washington	24	24						
Nevada	19	9		4	1	3		2
Connecticut	16	16						
Wyoming	9	2	4	2			1	
Total	6299	4883	571	467	149	124	80	25

*Arranged in order of number of analyses submitted.

TABLE IV

NUMBER OF COMMUNAL WATERS TESTED IN EACH STATE ARRANGED IN ORDER OF DIMINISHING MAGNITUDES. ALSO REVEALS TOTAL POPULATION OF EACH STATE AND THE NUMBER EXPOSED TO COMMUNAL WATERS WITH FLUORINE A NATURAL CONSTITUENT IN STRENGTHS FROM 0.5 TO 5.1 PPM AND HIGHER

STATES	COMMUNAL WATERS ANALYSED	POP. OF STATE	POP. ACCESS TO F. WATER	PER CENT	FLUORINE CONCENTRATIONS (PPM)				
					0.5 TO 0.8	0.9 TO 2.0	2.1 TO 3.0	3.1 TO 5.0	5.1 AND UP
Illinois	639	7,897,241	573,633	7.26	219,120	335,950	13,942	4,138	483
Texas	547	6,414,824	2,134,021	33.26	888,940	725,500	429,045	65,905	24,631
Ohio	438	6,907,612	298,079	4.30	115,365	150,213	32,501		
Minnesota	423	2,792,380	33,767	1.20	32,140	1,627			
Kansas	378	1,801,028	166,929	9.26	133,975	30,600		2,354	
Georgia	351	3,123,723	56,116	1.79	56,116				
Florida	317	1,897,414	280,039	14.75	206,615	72,587	837		
Wisconsin	283	3,137,587	163,535	5.21	100,148	10,779	52,608		
New Jersey	268	4,160,165	20,268	0.48	3,550	16,718			
Utah	251	550,316	11,384	2.00	9,111	1,983		290	
Indiana	248	3,427,796	276,048	8.05	205,633	68,967		1,448	
Missouri	220	3,784,664	154,074	4.07	96,299	51,612	2,414	3,749	
So. Dakota	204	642,961	115,921	18.02	61,209	16,901	24,357	10,703	2,751
Tennessee	190	2,915,841	27,658	0.94	15,637	12,021			
New Mexico	159	531,818	140,936	26.50	95,200	41,409	3,912	415	
Nebraska	146	315,834	58,877	18.64	24,009	35,235			
Michigan	141	5,256,106	95,461	1.81	88,301	7,160			
Idaho	136	524,873	57,000	10.85	40,094	16,550		356	
Maryland	133	1,821,244	27,523	1.51	10,837	11,674	1,104	3,908	
Arkansas	94	1,949,387	24,834	1.26	19,485		5,349		
Mississippi	93	2,183,796	11,849	0.42	3,445	8,404			
Louisiana	80	2,363,880	129,854	5.49	37,252	83,115	1,615		7,872
No. Dakota	70	641,935	82,164	12.79	21,022	35,235	3,102	22,544	261
Colorado	52	1,123,296	476,838	42.44	82,850	345,870	43,829	4,289	
R. Island	46	713,346	400	0.056		400			
Virginia	44	2,677,773	54,792	2.04	37,642	11,147	300	1,549	4,153
California	43	6,907,387	94,764	1.37	20,914	60,615	10,017	3,218	
Nevada	19	110,247	1,795	1.62		350	1,245	200	
Connecticut	16	1,709,242	30,456	1.78	30,456				
Wyoming	9	250,742	47,882	19.09	2,845	42,860		2,177	
Totals	6038		5,646,897		2,658,211	2,195,115	626,177	127,243	40,151

TABLE V

TOTAL NUMBER OF PEOPLE IN FIVE STATES HAVING ACCESS TO COMMUNAL WATER CONTAINING FLUORINE CONCENTRATIONS OF 0.5 PPM AND HIGHER

STATES	COUNTIES	POP. OF STATE	POP. EXPOSED TO FLUORINE	FLUORINE CONCENTRATIONS (PPM) 0.5 TO 0.8	0.9 TO 2.0	2.1 TO 3.0	3.1 TO 5.0	5.1 AND UP
Illinois	60	7,897,241	573,633	219,120	335,950	13,942	4,138	483
Indiana	44	3,427,796	276,048	205,633	68,967	32,501	1,448	
Ohio	44	6,907,612	298,079	115,365	150,213			
South Dakota	52	642,961	115,921	61,209	16,901	24,357	10,703	2,751
Texas	185	6,414,824	2,134,021	888,940	725,500	429,045	65,905	24,631

TABLE VI

TOTAL NUMBER OF PEOPLE AND NUMBER OF COMMUNITIES IN FIVE STATES HAVING ACCESS TO COMMUNAL WATERS WITH FLUORINE CONCENTRATIONS TESTING FROM 0.0 TO 5.1 PPM AND HIGHER

STATES	COMMUNAL WATERS ANALYSED	POP. OF STATE	FLUORINE CONCENTRATIONS (PPM) 0.0 TO 0.5	0.6 TO 0.9	1.0 TO 1.5	1.6 TO 2.0	2.1 TO 3.0	3.1 TO 5.0	5.1 AND UP
Illinois	639	7,897,241	5,565,785	151,295	148,037	146,587	13,942	4,138	483
Cities and towns			480	77	48	19	10	4	1
Indiana	248	3,427,796	621,663	126,682	50,074	12,626		1,448	
Cities and towns			174	42	25	6		1	
Ohio	438	6,907,612	3,664,459	103,348	82,652	25,992	32,501		
Cities and towns			319	41	49	22	7		
South Dakota	204	642,961	179,557	43,156	11,692	2,081	24,357	10,703	2,751
Cities and towns			84	32	23	5	37	18	5
Texas	547	6,414,824	740,013	706,928	303,442	147,004	429,045	65,905	24,631
Cities and towns			219	99	125	35	41	19	9

TABLE VII

6299 COMMUNITIES AND NUMBER OF PEOPLE IN THIRTY-TWO STATES HAVING ACCESS TO COMMUNAL WATER WITH FLUORINE CONCENTRATIONS TESTING FROM 0.0 TO 5.1 PPM AND HIGHER

	FLUORINE CONCENTRATIONS (PPM)			
	0.0 TO 0.5	0.6 TO 0.99	1.0 TO 1.5	1.6 TO 2.0
Population	32,072,330	1,863,310	1,293,915	920,671
Communities	4,883	571	467	149
	2.1 TO 3.0	3.1 TO 5.0	5.1 AND HIGHER	
Population	626,177	127,243	40,151	
Communities	124	80	25	

TABLE VIII

POPULATION OF THIRTY STATES AND NUMBER OF PEOPLE HAVING ACCESS TO WATER CONTAINING FLUORINE IN CONCENTRATIONS OF 0.5 TO 5.1 PPM AND HIGHER

WATER ANALYSES SUBMITTED	TOTAL POPULATION	POP. ACCESS TO F. WATER	PER CENT
6038	78,534,458	5,646,897	7.24

	FLUORINE CONCENTRATIONS (PPM)				
	0.5 TO 0.8	0.9 TO 2.0	2.1 TO 3.0	3.1 TO 5.0	5.1 AND HIGHER
Population	2,658,211	2,195,115	626,177	127,243	40,151

FINDINGS

We find from the figures presented to us that fluorides occur as a natural constituent of some of the communal water supplies in thirty-two states (Tables II, III, IV, VII). Three areas composed of (1) Indiana, Ohio, Illinois, (2) the South Dakota area, and (3) the state of Texas, show a heavier concentration of communal water containing fluorides than any other portion of the country (Tables V, VI). In order to compare the fluorine distribution in these five states with their respective populations, each state has been broken down into counties as is shown in Figs. 1 to 6. In Illinois there are four communities using water containing fluorides in a concentration of 3.1 to 5.0 ppm, with an over-all population of 4,138. In Texas there are nineteen communities using fluorinated water of the 3.1 to 5.0 ppm concentration with an over-all population of 65,905 (Table IX). There are nine states containing twenty-five communities with a known population of 40,151 that are consuming water which contains 5.1 or more parts per million of fluorine (Table X).

TABLE IX

COMMUNAL WATER SUPPLIES CONTAINING FLUORINE IN STRENGTHS FROM 3.1 TO 5.1 PPM AND HIGHER

COMMUNITIES	FLUORINE CONCENTRATIONS 3.1 TO 5.0 PPM	POPULATION
Texas		
Farmers Branch	3.2	Not listed
Arcadia Park	3.4	Not listed
Mesanite	4.0	Not listed
Pilot Point	3.8	1,122
Pendleton	3.6	Not listed
Seminole	4.0	1,761
Hale Center	3.4	836
Plainview	3.3	8,263
Levelland	3.5	3,091
Tahoka	4.0	2,129
Idalou	3.2	503
Slaton	4.4	3,587
Ballinger	4.2	4,472
Marshall Ford	3.4	Not listed
Cedar Valley	3.5	Not listed
Brownfield	3.2	4,009
Georgetown	3.6	3,682
Hutte	4.0	597
Lubock	4.5	31,853
Total		65,905
South Dakota		
Agar	3.6	142
Capa	5.0	271
Claremont	4.5	275
Columbia	4.0	395
Conde	3.2	542
Doland	3.2	1,457
Eureka	3.2	85
Ferney	5.0	100
Kimball	3.2	997
Lake Preston	5.0	886
Langford	5.0	452
Northville	4.5	223
Oldham	5.0	386
Pukwana	3.2	258
Redfield	3.4	2,428
Rockham	3.2	220
St. Lawrence	3.2	297
Tyndall	3.2	1,289
Total		10,703
North Dakota		
Casselton	5.0	1,358
Columbus	3.6	506
Devils Lake	4.0	6,204
Ellendale	3.2	1,517
Elliott	3.2	118
Goodrich	3.2	476
Grafton	3.6	4,070
Hettinger	3.2	1,138
Leeds	3.6	782
Mott	3.2	1,220
Wahpeton	4.0	3,747
Park River	4.0	1,408
Total		22,544

TABLE IX—CONT'D

COMMUNITIES	FLUORINE CONCENTRATIONS 3.1 TO 5.0 PPM	POPULATION
California		
Twentynine Palms	4.0	1,218
Death Valley	3.4	Not listed
Death Valley Jn.	4.0	Not listed
Keeler	3.0	Not listed
Desert Center	5.0	Not listed
Monticeto	3.2	2,000
Hot Springs'	4.5	Not listed
Total		3,218
Illinois		
Kempton	3.6	259
Ipava	4.4	629
Biggsville	3.6	344
Bushnell	4.0	2,906
Total		4,138
New Mexico		
Columbus	4.0	265
Radium Springs	3.4	100
Rosebud	3.4	50
Total		415
Virginia		
Smithfield	3.2	1,178
Bellwood	5.0	Not listed
Holland	3.6	371
Total		1,549
Kansas		
Dighton	3.2	947
La Cross	3.4	1,407
Total		2,354
Missouri		
Concordia	3.14	1 077
Vandalia	3.3	2,672
Total		3,749
Louisiana		
Grayson	3.1	Not listed
Urania	4.1	Not listed
Colorado		
Brighton	3.5	4,029
Olney Springs	3.5	260
Total		4,289
Indiana		
Lowell	4.5	1,448
Utah		
Tridell	3.6	290
Idaho		
Newdale	5.0	356
Maryland		
Criesfield	5.0	3,908
Wyoming		
Gillette	4.5	2,177

TABLE X

COMMUNAL WATER SUPPLIES CONTAINING FLUORINE IN STRENGTHS FROM 5.1 PPM AND HIGHER

COMMUNITIES	FLUORINE CONCENTRATIONS 5.1 PPM	POPULATION
Texas		
Lamesa	5.2	6,038
Ackerly	6.8	Not listed
Belton	8.5	3,572
Clifton	8.0	1,732
New Braunsfels	5.6	6,976
Crosbytown	7.2	1,615
Ratan	5.5	2,209
Sierra Blanca	5.2	821
Bartlett	6.7	1,668
Total		24,631
South Dakota		
Bath	6.0	128
Britton	7.6	1,500
Hecla	5.5	555
Pierpont	5.5	362
Raymond	5.5	206
Total		2,751
Virginia		
Eastville	9.1	316
Franklin	5.8	3,466
Holland	5.6	371
Total		4,153
Louisiana		
Bunkie	6.8	3,575
Pineville	7.4	4,297
Total		7,872
North Dakota		
Regent	6.0	261
Nevada		
Beatty	7.6	200
Gabbs	6.5	Not listed
California		
Benton	9.0	Not listed
Mississippi		
Belmont	5.1	Not listed
Illinois		
Bureau Junction	5.5	483

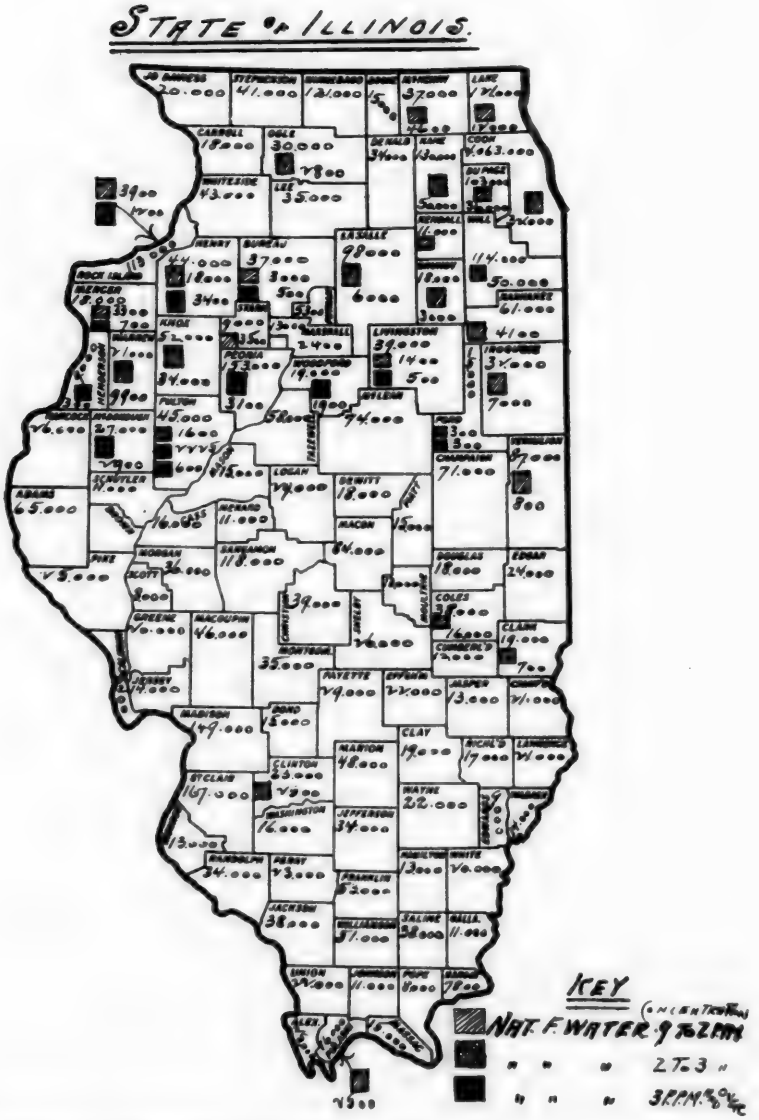

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Fig. 1.—Number of people in counties of Illinois having access to naturally fluoride-bearing water. Largest figure is county population. (Published by permission.)

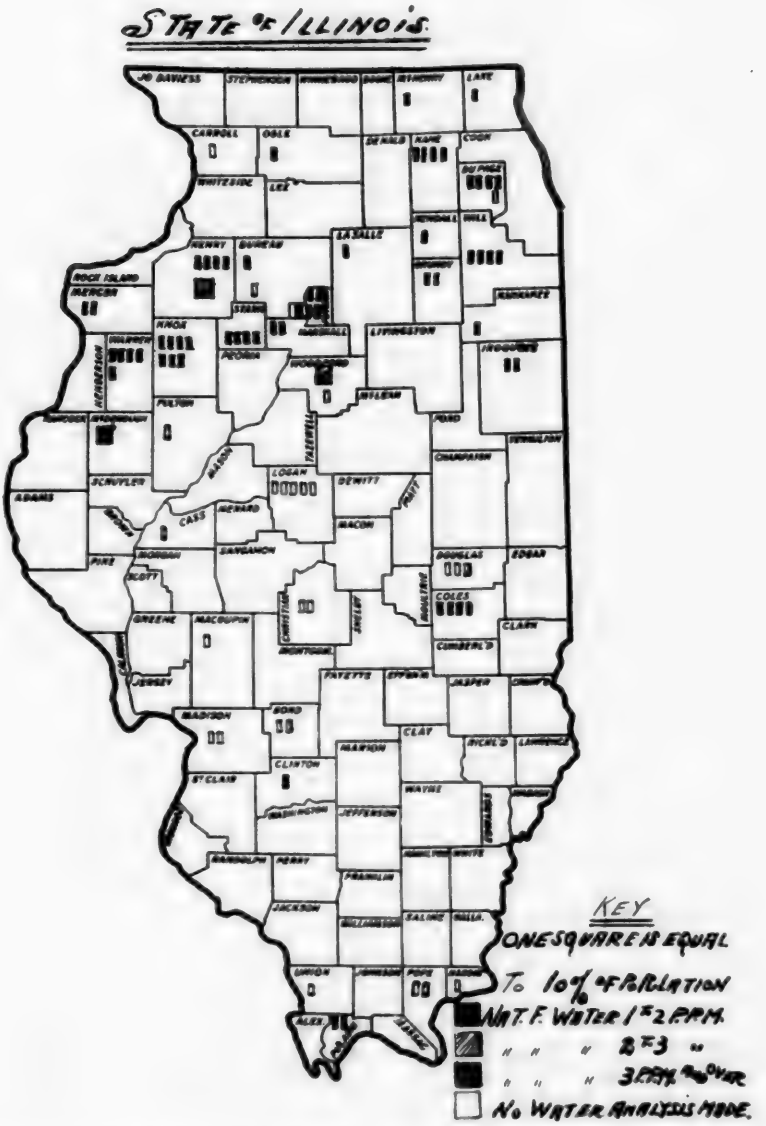
STATE OF ILLINOIS
KEY
ONE SQUARE IS EQUAL
TO 10% OF POPULATION
WAT. F. WATER 1 TO 2 P.P.M.
" " " 2 TO 3 "
" " " 3 P.P.M. AND OVER
NO WATER ANALYSIS MADE.

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Fig. 2.—Percentage of population of each county in Illinois having access to water naturally containing fluorides. The approximate location of fluoride-bearing water in Illinois is shown. (Published by permission.)

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Fig. 3.—Number of people in counties of Indiana using naturally fluoride-bearing water. Largest figure is county population. (Published by permission.)

DISCUSSION

In the past few years some reports have raised the question as to the incidence of certain systemic and oral diseases which are thought to be attributed to the consumption of water containing fluorine. It is not the purpose of this study to question the validity of such reports. However, knowing the areas in

Fig. 4.—Number of people in counties of Ohio having access to naturally fluoride-bearing water. Largest figure is county population. (Published by permission.)

the United States which have fluoride-bearing waters as well as the number of people in each community who consume such water (Tables II, III), we will have provided the opportunity to study the vital statistics of each county and state in an effort to determine if the incidence of certain diseases is higher in the

areas containing fluorine than in the nonfluorine areas. Knowing these specific fluorine communities, we have an aid in evaluating a study where the artificial fluorination of water has been made. These data will provide the opportunity for comparing the caries incidence of children from an endemic area with the caries incidence of those from a nonendemic area at the time they move into a community in which a study program is under way.

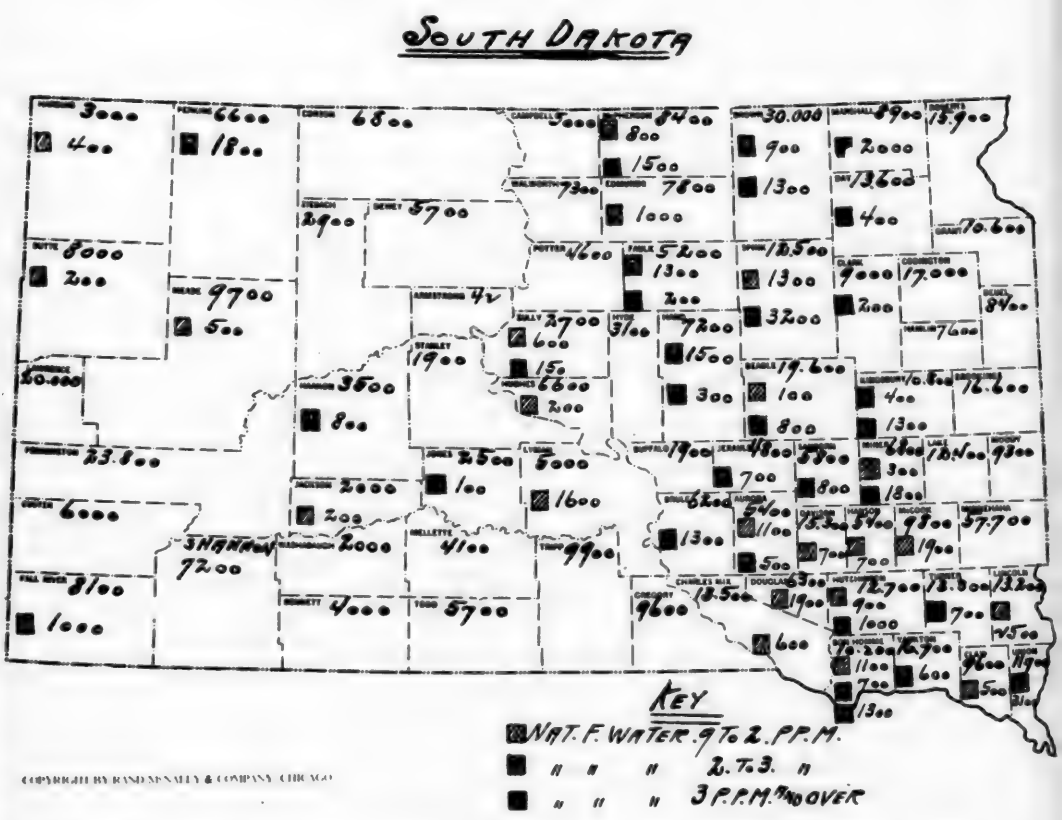

Fig. 5.—Number of people in counties of South Dakota having access to naturally fluoride-bearing water. Largest figure is county population. (Published by permission.)

It is acknowledged that the incidence of dental caries in a community whose water supply has fluorine as a natural constituent is less than the caries incidence of a community whose water supply is fluorine free.[6] The per cent of hearing defects due to otosclerosis and fibrosis according to Lewy[7] is lower in an area having a water containing fluorine than in an area where the water is fluorine free. If the addition of fluorides to communal water supplies proves to be successful, then communities with naturally fluorinated water should either reduce or raise the fluorides to the optimal concentration, thus insuring the best result. The question arises "How many communal water supplies have

had the excess fluorine removed?'' Maier in his article, ''Methods of Removing Fluorides From Water,''[8] says no community has yet attempted to treat the water for the excess fluorine and subsequently measure the results in terms of a reduction of the incidence of fluorosis.

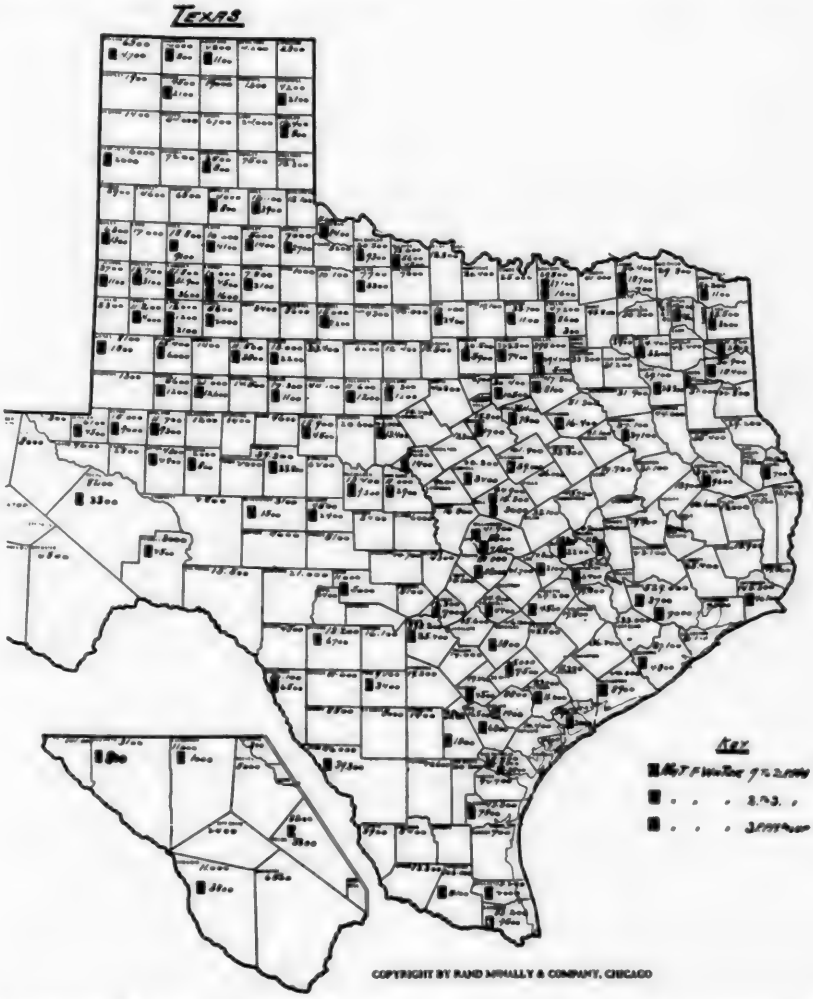

Fig. 6.—Number of people in counties of Texas having access to naturally fluoride-bearing water. Largest figure is county population. (Published by permission.)

SUMMARY

The municipalities having a high fluorine content are found in nine states. with the greatest number in Texas and the next greatest number in South Dakota. There are approximately 5,646,897 people in the United States having access to a communal water supply containing fluorine in the amount of 0.5 ppm or more (Table VIII). This is approximately 4.307 per cent of the entire population of the United States. A total of 2,195,115 people are in the fluorine

concentration bracket of 0.9 to 2.0 parts per million. In the 2.1 to 3.0 ppm bracket, there are 626,177 persons. In a still higher concentration of 3.1 to 5.0 ppm, there are 127,243 people (Table IX). Municipalities of high fluorine content, 5.1 ppm and higher, number only twenty-five and the number of people exposed to a high level of fluorine is not great, namely, 40,151. More detailed water analyses should be conducted to determine the fluorine content of water supplied to children during the years in which tooth formation is taking place.

The communities in the high fluorine areas, though few in number and low in population, should either (1) consider changing the source of their water supply, (2) remove excess fluorine by chemical means, or (3) reduce the fluorine content by adding water of lower fluorine concentration to arrive at a level which is not harmful and yet will provide protection against tooth decay.

The authors wish to thank Dr. W. H. Tucker for his advice and criticism during this work.

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GEOGRAPHIC VARIATIONS OF DENTAL CARIES IN OREGON

I. Dental Status of Native Born and Reared School Children in Two Regions*

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WITHIN the last few years the epidemiological study of dental caries has received great attention from a number of investigators here as well as abroad. From numerous reports on this subject in the literature during the past decade, valuable data have been presented which when they are put together tend to create a pattern which reveals some of the characteristics peculiar to dental caries attack and also some of the factors which do or may have a bearing on caries resistance. In a preliminary investigation on the incidence of dental caries among freshman students at Oregon State College, it was reported[1] that the D.M.F. rate was high as compared with that of similar age groups in other geographic areas of the country, and indications were that some areas within the state might have higher incidence of the disease than others.

Two geographic areas within the state, namely, the Coast and Central Oregon regions, were selected as representing a high and a low area of caries incidence. The investigation reported in this paper was conducted to find the dental caries attack rate of a selected group of people of these areas, and also some of the factors that might influence it.

This paper deals only with the dental findings, and a forthcoming paper will present and discuss some of the environmental factors which may have an influence on the rate of attack.

MATERIAL AND METHOD

Dental examinations were conducted in two counties in each of the geographic areas. In the Coast region the counties of Clatsop and Coos were investigated, and in the Central Oregon region the counties of Deschutes and Klamath. These counties were selected on the basis of their better local health facilities and organization, and also because they were more densely populated as compared with the other counties within the two regions.

The subjects of the investigation were white school children 14, 15, and 16 years of age. In order to have a homogeneous group, the dental examination was given only to those children who were native born and reared. A child

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was classified as native born and reared if, during the first eight years of his life, no break occurred in continuity of residence within his native county which totaled more than one month in any one calendar year, and also if after his eighth birthday and up to the time of the examination he was not absent from the county for a length of time which totaled more than three months in any calendar year. Children meeting the above requirements were examined from almost all of the schools in each one of the four counties visited. A public health nurse made the contacts with the schools and explained to the staff the purpose of the investigation. The eligibility of the subjects was determined in the classroom. At the time of the examination the child was again questioned regarding previous residence. The total number of children examined in any one day was not more than twenty-four, and on the average a period of fifteen minutes was spent for each child.

The dental examination was done by means of mouth mirror and explorers in front of a small window in a trailer. A dental spotlight was used for auxiliary light. Posterior bite-wing radiographs, one for each side, were taken of most of the children. The dental findings were called out to a dental assistant who made appropriate markings on a dental record. Items for which observations were made included: (1) number of filled teeth; (2) number of missing teeth; (3) number of carious teeth indicated for filling; (4) number of carious teeth indicated for extraction; (5) number of carious surfaces indicated for filling detected by clinical examination, and (6) number of carious surfaces indicated for filling detected by radiographs. A separate record was set up for observations of cases of hypoplasia.

All examinations were made by the senior author, a fact which adds uniformity to the collected data, as the method employed for the examination of all the subjects was the same.

The carious lesions recorded were those which can be usually observed on a careful clinical examination. A grayish white or white spot on the enamel was considered a carious lesion if the explorer caught when passed lightly over the surface. Pits and fissures were listed as carious if the explorer caught and only when upon careful examination a carious lesion was actually found. Observations were not made for third molars and deciduous teeth, a few of which were encountered. All missing teeth were assumed to have been lost because of extensive caries except in a few cases where the subject upon questioning stated other reasons, for example, lost by accident or extracted because of orthodontic treatment. In these instances, the missing teeth were not listed.

The dental caries experience was measured by counting the number of filled teeth, teeth missing because of caries, teeth indicated for extraction, and teeth with untreated dental caries indicated for filling. The symbol D.M.F. was used to designate past and present caries experience for teeth and tooth surfaces.

FINDINGS

The material presented in Table I gives information on the percentage of the total number of school children, by sex, in the three combined age groups of the four counties under investigation who met the standards set for eligibility

in the examination, and also on the percentage of the eligible ones examined. As it can be seen, the percentage of children eligible for examination is rather similar in the four counties. Furthermore, the number of eligible children of both sexes given the examination is well over 50 per cent of the total in all the counties with the exception of boys in Deschutes county. These two factors greatly strengthen the homogeneity of the subject population and the significance of the findings.

The total number of children examined was 741. Their distribution by age, sex, county, and geographic region is given in Table II. The number of subjects by sex in all age groups was about evenly divided in the counties of Clatsop, Coos, and Klamath. In Deschutes county the ratio of girls to boys was two to one.

The data shown in Table III reveal the prevalence of dental caries experience (D.M.F.) in terms of teeth and tooth surfaces per child by specified age groups. The figures given in this table and subsequent tables are exclusive of the carious lesions detected by posterior bite-wing radiographs. The average number of D.M.F. teeth and tooth surfaces increases simultaneously with the chronological age of the subjects. This is a characteristic peculiar to dental

TABLE I

PERCENTAGE OF NATIVE BORN AND REARED SCHOOL CHILDREN ELIGIBLE FOR EXAMINATION AND PERCENTAGE EXAMINED BY SEX IN THE THREE AGE GROUPS COMBINED

REGION	COUNTY	SEX	PER CENT ELIGIBLE	PER CENT EXAMINED
Coast	Clatsop	Boys	32	55
		Girls	27	59
		Both sexes	29	57
	Coos	Boys	34	68
		Girls	34	66
		Both sexes	34	67
Central Oregon	Deschutes	Boys	22	39
		Girls	27	65
		Both sexes	24	53
	Klamath	Boys	24	61
		Girls	24	64
		Both sexes	24	62

caries attack, and it has been found that the rate of increase varies with different population groups depending upon several extrinsic and intrinsic factors.

As it can be seen from the table, the dental caries experience of the subjects in all age groups varied from county to county. The average number of D.M.F. teeth of the 14-year-old child in Clatsop county was 13.3 while his counterpart in Coos county was 11.1, a difference of two D.M.F. teeth. The same difference was observed in the 14-year-old children between the counties of Deschutes and Klamath of the Central Oregon region. On the other hand, a difference of 1.7 D.M.F. teeth was observed between the rates of all ages of Clatsop and Coos counties, while the difference in the counties of Deschutes and Klamath between the rates of all ages rises to 2.3 D.M.F. teeth. Moreover, if we take the D.M.F. teeth rate of the 14-year-old in Clatsop county of the Coast region, which was 13.3, and compare it with that of his counterpart in Klamath county of the Central Oregon region, which was 7.5, we observe a

TABLE II

AGE AND SEX DISTRIBUTION OF 741 NATIVE BORN AND REARED SCHOOL CHILDREN EXAMINED IN TWO GEOGRAPHIC REGIONS OF OREGON

REGION	COUNTY	SEX	AGE IN YEARS (LAST BIRTHDAY) 14	15	16	ALL AGES
Coast	Clatsop	Boys	37	23	32	92
		Girls	35	30	20	85
		Both sexes	72	53	52	177
	Coos	Boys	47	55	27	129
		Girls	46	34	33	113
		Both sexes	93	89	60	242
Central Oregon	Deschutes	Boys	11	10	16	37
		Girls	25	23	23	71
		Both sexes	36	33	39	108
	Klamath	Boys	36	37	29	102
		Girls	39	42	31	112
		Both sexes	75	79	60	214
Both	All	Boys	131	125	104	360
		Girls	145	129	107	381
		Both sexes	276	254	211	741

TABLE III

NUMBER OF D.M.F. PERMANENT TEETH AND TOOTH SURFACES OF 741 NATIVE BORN AND REARED SCHOOL CHILDREN BY SPECIFIED AGE GROUPS IN TWO GEOGRAPHIC REGIONS OF OREGON. RATES ARE EXPRESSED PER CHILD

REGION	COUNTY	PERMANENT TEETH	AGE IN YEARS (LAST BIRTHDAY) 14	15	16	ALL AGES
Coast	Clatsop	D.M.F. teeth	13.3	15.1	15.3	14.4
		D.M.F. surfaces	30.1	35.8	36.4	33.6
	Coos	D.M.F. teeth	11.1	13.6	13.9	12.7
		D.M.F. surfaces	24.0	30.6	30.7	28.1
Central Oregon	Deschutes	D.M.F. teeth	9.6	12.0	12.3	11.3
		D.M.F. surfaces	21.4	25.5	28.2	25.1
	Klamath	D.M.F. teeth	7.5	9.6	10.0	9.0
		D.M.F. surfaces	15.2	19.2	20.7	18.2

difference of 5.8 D.M.F. teeth. A similar difference is observed in the remaining two age groups of these two counties. The same wide variability in the number of D.M.F. tooth surfaces is also present among the school children of the two geographic regions. The following number of children were found to be caries free in the four counties: Clatsop, none; Coos, three; Deschutes, three; and Klamath, eight. The D.M.F. teeth rate of all ages of every county was compared with each one of the other three and the difference was found to be statistically significant. The differences in the combined rates of all ages between the two regions (3.7 D.M.F. teeth) was also statistically significant.

From all the above findings it is shown that a difference of varying degree exists in the incidence of dental caries among school children in two geographic areas of the State of Oregon. The more pronounced disparity is observed between Clatsop county of the Coast region and Klamath county of the Central Oregon region.

In order to compare the data of this investigation with the findings of surveys conducted in other parts of the country, Table IV was prepared. As it can be seen, the average number of D.M.F. teeth of the school children of all age groups in Clatsop county, Oregon, was more than twice as much as that of

the school children of the same age groups in Hagerstown, Md., San Francisco, and New York City.[2] Progressively smaller differences were noted between the other three counties of Oregon and the three places mentioned, the least one being of that in Klamath county where, on the average, a difference of more than two D.M.F. teeth was observed. However, it should be remembered that for the children examined in the three other localities of the country no standards were set regarding history of previous residence.

The relationship between sex and caries prevalence has been pointed out in the past during studies of different population groups.[1, 3, 4] The D.M.F. teeth and tooth surfaces of the 741 school children were calculated by age and sex in the four counties under investigation and the rates are shown in Table V. It is apparent from this table that in general boys experienced a lower rate of attack than girls of the same chronological age. This trend has been attributed to differences in posteruptive tooth age between boys and girls,[5] that is, the teeth of girls in general erupt earlier than those of boys and they are exposed longer to the factors responsible for the occurrence of the carious lesions.

Another point of interest which was observed from this table is a tendency in both sexes for a considerable increase in the rates of the caries experience between the ages fourteen to fifteen as compared with that of the age groups fifteen to sixteen. Although a single examination does not show when dental caries occurred, this phenomenon can possibly be explained as due to the increased susceptibility to dental caries during the period of early adolescence. Such an increase can be measured accurately in longitudinal studies of individual cases carried over a period of years, because when large numbers of children are considered, a smooth curve is usually obtained which obliterates the peaks of caries activity. Previous studies by Hanke,[6] Noyes,[7] and Massler[8] have also shown a rapid rise in the formation of cavities during the early teen ages. It is generally agreed by these investigators that the increased susceptibility is associated with the adolescent phase of rapid growth which precedes puberty, and constitutes a problem of special clinical consideration. The data presented in Table V seem to confirm this observation.

TABLE IV
COMPARISON OF AVERAGE NUMBER OF D.M.F. TEETH PER PERSON BY SPECIFIED AGE GROUPS OF FOUR COUNTIES IN OREGON, HAGERSTOWN, MD., SAN FRANCISCO, AND NEW YORK CITY*

LOCALITY	AGE IN YEARS (LAST BIRTHDAY)		
	14	15	16
Clatsop, Ore.	13.3	15.1	15.3
Coos, Ore.	11.1	13.6	13.9
Deschutes, Ore.	9.6	12.0	12.3
Klamath, Ore.	7.5	9.6	10.0
Hagerstown, Md.	5.6	6.6	7.2
San Francisco	5.6	6.5	7.2
New York City	5.8	6.9	7.4

*History of previous residence of the children was not taken into account for eligibility in the examination in Hagerstown, Md., San Francisco, and New York City.

A detailed presentation of the four items comprising the dental caries experience of the subjects by specified age and sex groups is given in Table VI. The teeth of girls have received better care, although, as it was shown in Table V, they have experienced higher rates of D.M.F. teeth and tooth surfaces than

TABLE V
NUMBER OF D.M.F. PERMANENT TEETH AND TOOTH SURFACES OF 741 NATIVE BORN AND REARED SCHOOL CHILDREN BY SPECIFIED AGE AND SEX GROUPS IN TWO GEOGRAPHIC REGIONS OF OREGON. RATES ARE EXPRESSED PER CHILD.

REGION	COUNTY	PERMANENT TEETH	BOYS AGE IN YEARS (LAST BIRTHDAY)				GIRLS AGE IN YEARS (LAST BIRTHDAY)			
			14	15	16	ALL AGES	14	15	16	ALL AGES
Coast	Clatsop	D.M.F. teeth	12.3	12.7	15.6	13.5	14.2	17.0	15.0	15.4
		D.M.F. surfaces	28.7	27.3	37.0	31.2	31.5	42.3	35.4	36.2
	Coos	D.M.F. teeth	10.4	13.3	13.5	12.3	11.7	14.1	14.2	13.2
		D.M.F. surfaces	21.5	29.5	27.6	26.2	26.5	32.3	33.2	30.2
Central Oregon	Deschutes	D.M.F. teeth	7.4	10.0	10.3	9.3	10.6	12.9	13.7	12.3
		D.M.F. surfaces	17.0	23.0	23.0	21.2	23.3	26.7	31.5	27.1
	Klamath	D.M.F. teeth	6.3	9.9	10.1	8.7	8.6	9.2	10.0	9.2
		D.M.F. surfaces	13.0	20.6	20.3	17.9	17.2	17.9	21.0	18.5

the boys. In girls, generally, the number of missing teeth and teeth requiring extraction because of extensive caries is similar to that of boys, at the same time the number of carious teeth and tooth surfaces requiring filling is less. This means that girls give earlier attention to their dental needs, and therefore, more teeth are saved by fillings. The same tendency was observed among the freshman girl students examined at Oregon State College,[1] and the only explanation that can be offered is that girls generally are more conscious of their dental needs for esthetic reasons.

Another observation of special interest in the two regions is the degree of dental care which had been received by the children of the four counties. Although the subjects of Clatsop county had higher rates of caries experience than those of Coos county they, nevertheless, showed fewer carious teeth and tooth surfaces requiring filling, whereas the opposite would have been expected. Similarly, the children of Deschutes county who experienced higher rates of caries attack showed fewer carious teeth and tooth surfaces in need of filling than were observed among those children of Klamath county with lower rates.

Of the 741 children examined, posterior bite-wing radiographs, one for each side, were taken of 505 of them. Table VII shows that, on the average, each child had 9.9 carious surfaces which needed filling. Of those, 7.4 were detected by explorer alone and 2.5 by taking posterior bite-wing radiographs. In other words, 25.3 per cent of the carious surfaces requiring filling would have been missed if radiographic examination had not been included. This percentage seems high, but the practicability of including radiographic examinations in epidemiological surveys on dental caries for the purpose of determining the dental status of population groups can be questioned. It has been shown in previous studies[1, 9] that radiographs, in addition to a careful examination by mirror and explorer, do not materially change the results of such surveys.

As a part of the dental examination of the school children, observations for cases of enamel hypoplasia were included. It has been reported in the literature of recent years[10] that hypoplasia is not restricted to certain geo-

TABLE VI

DENTAL CARIES EXPERIENCE OF 741 NATIVE BORN AND REARED SCHOOL CHILDREN IN TWO GEOGRAPHIC REGIONS OF OREGON. RATES ARE EXPRESSED PER CHILD BY SPECIFIED AGE AND SEX GROUPS

REGION	COUNTY	PERMANENT TEETH	BOYS AGE IN YEARS (LAST BIRTHDAY)				GIRLS AGE IN YEARS (LAST BIRTHDAY)			
			14	15	16	ALL AGES	14	15	16	ALL AGES
Coast	Clatsop	Filled	7.2	7.4	9.8	8.2	9.9	11.9	10.7	10.8
		Missing	0.7	1.0	1.1	0.9	0.8	1.7	1.0	1.2
		Extraction indicated	0.2	0.3	0.5	0.3	0.1	0.2	0.1	0.2
		Filling indicated	6.2	5.3	6.6	6.1	5.2	6.2	5.8	5.7
		Surfaces indicated for filling	9.3	7.5	9.3	8.8	7.5	8.4	8.6	8.1
	Coos	Filled	4.6	7.7	6.3	6.3	6.6	9.5	9.1	8.2
		Missing	0.6	0.7	1.0	0.7	0.6	1.0	1.0	0.8
		Extraction indicated	0.3	0.3	0.2	0.3	0.3	0.1	0.2	0.2
		Filling indicated	6.1	6.6	7.5	6.6	6.8	6.0	5.9	6.3
		Surfaces indicated for filling	8.6	9.5	10.3	9.3	9.5	8.5	8.8	9.0
Central Oregon	Deschutes	Filled	3.4	6.6	7.1	5.9	7.8	9.9	8.6	8.7
		Missing	0.7	1.0	1.1	0.9	0.8	0.9	1.2	1.0
		Extraction indicated	0.3	0.2	0.1	0.2	0.2	0.0	0.1	0.1
		Filling indicated	3.7	3.5	3.1	3.4	2.9	3.7	5.0	3.9
		Surfaces indicated for filling	5.5	4.5	4.4	4.7	3.8	4.5	7.1	5.1
	Klamath	Filled	2.4	5.5	4.2	4.0	4.9	5.0	4.8	4.9
		Missing	0.8	1.1	0.8	0.9	0.6	0.6	1.0	0.7
		Extraction indicated	0.2	0.2	0.1	0.1	0.2	0.1	0.5	0.3
		Filling indicated	3.6	4.4	6.0	4.5	3.9	4.3	4.5	4.3
		Surfaces indicated for filling	5.0	6.3	9.1	6.6	5.7	6.1	6.1	6.0

graphic locations, as mottled enamel is, but it is ubiquitous in its occurrence and that, also, because of its high incidence it constitutes a public health problem which needs further study. Sterling[11] found an incidence of 2.5 per cent among Negro school children. Sarnat and Schour[10] stated that enamel hypoplasia occurs in approximately 5 per cent of the school children of Chicago. Mellanby[12] examined children of Finnish Lapps and reported hypoplastic lesions in 3.9 per cent of them. Brucker[13] placed its incidence at 4 per cent among school children in Newark, New Jersey. In the present investigation of 741 school children, no cases of enamel hypoplasia were observed. For this, no explanation can be advanced at the present time.

COMMENTS

The data presented in this paper supply another link to the chain of epidemiological studies on dental caries experience among selected population groups. In most of the epidemiological studies on dental caries of school children in different parts of the country, no attempt was made to set up standards relative to previous residence of the subject population for eligibility in the examination. Notable exceptions are the studies of the fluorine-dental caries relationship[14, 15, 16] where only those children continuously exposed (native born and reared) to the common water supply were examined. In the present investigation we followed closely the procedure employed in the above mentioned studies, and the examination was given only to those children who were classi-

fied as native born and reared. The main difference was that in this study the whole county was considered as the place of residence, while in the studies of fluoride domestic water and the prevalence of dental caries, the unit of residence was the city. The classification of school children as native born and reared on a county basis has some apparent shortcomings which are excluded when the city is considered as residence. Nevertheless, because of the elimination of the "circulation factor" to a certain degree, the findings obtained have a greater significance as compared with those of investigations where no consideration was given to the subject's previous residence. This is particularly true in the case of the states of the Pacific Coast where the current of westward migration in the last few years was considerable. Studies as the present one offer better possibilities for clarification of the role played by some of the environmental and other factors in caries attack and resistance. The data gathered can be of more value where no appreciable amount of fluorine is present in the common water supplies, and yet a distinct variation in caries attack exists between two regions. In the present investigation water samples were taken from different towns and cities located in the four counties and their analyses will be presented and discussed in another paper.

TABLE VII

COMPARISON OF CARIOUS SURFACES REQUIRING FILLING DETECTED BY MEANS OF CLINICAL AND X-RAY EXAMINATIONS OF 505 NATIVE BORN AND REARED SCHOOL CHILDREN IN TWO GEOGRAPHIC REGIONS OF OREGON. RATES ARE EXPRESSED PER CHILD

TOTAL NUMBER	NUMBER DETECTED BY EXPLORER	NUMBER DETECTED BY RADIOGRAPH	PER CENT DETECTED BY RADIOGRAPH
9.9	7.4	2.5	25.3

The findings of the present study reveal that the magnitude of the dental caries problem among school children living in two geographic areas of the same state varies considerably. From a public health point of view this fact seems to indicate that it is highly advisable and necessary to do further research in all the parts of the state with the thought of finding the prevalence of the disease in different geographic regions. As a result of such an investigation, areas of high rates of caries attack should be considered first for the application of preventive and corrective measures that are deemed necessary. Particularly this is true in view of the fact that existing facilities and personnel for public health dentistry are not adequate in most of the cases. Moreover, such an investigation will serve as a basis for appraising from time to time the effectiveness of the preventive and corrective measures which are used.

SUMMARY

Data from dental records of 741 native born and reared white school children of 14, 15, and 16 years of age in two geographic regions of Oregon have been analyzed and the findings reveal that:

1. There exists a difference of varying degree in the incidence of dental caries among school children living in two areas of the state, and that the more pronounced one was observed between Clatsop county of the Coast region and Klamath county of the Central Oregon region.

2. The dental caries experience of school children in both regions was found to be higher in comparison with that of subjects of the same age groups examined in other parts of the country.

3. In general, boys experienced a lower rate of caries attack than girls of the same chronological age.

4. The teeth of girls had received greater dental care although their dental caries attack rate was higher than that of boys.

5. Posterior bite-wing radiographs were taken of 505 subjects. They revealed 25.3 per cent of all the carious surfaces requiring filling in these children.

6. No cases of enamel hypoplasia were discovered among the school children examined in both areas.

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THE EFFECTS OF CARIES-PRODUCING DIETS INITIATED AT VARIOUS STAGES OF PRE- AND POSTNATAL DEVELOPMENT OF THE HAMSTER

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MANY workers have reported that the dental caries susceptibility of individuals may be influenced by certain nutritional or dietary factors during the time of the development of the teeth.[1-4] Sognnaes has more recently reported that the Syrian hamster developed more caries when a caries-producing diet based on a high carbohydrate content was initiated before tooth development than when initiated after the teeth were fully formed.[5]

In the course of a number of experiments in this laboratory, it was found that dental caries was not developing in the hamster to the extent nor with the rapidity expected on the basis of other reports.[6, 8] Since the animals here usually were not started on a caries producing diet (CPD) until weaning (21 days) when the first and second molars are already in occlusion, it was suspected that the diet should be initiated before tooth development in order to induce a greater caries incidence.

One other factor which we felt to be of importance was the fluorine content of the stock diet. When the stock diet is removed at the time of initiation of the CPD, the fluorine present in the former ration is, of course, no longer available and thus a potentially protective mechanism is lost if the fluorine is present in a biologically active state. Considering these points, the following experiments were designed. A total of one hundred twenty-one hamsters was studied.

EXPERIMENTAL

Part IA.—The offspring of eleven female hamsters were used. The mothers and/or the offspring were removed from the stock diet (Purina Laboratory Chow‡) and received a caries-producing diet§ at various pre- and postnatal stages of development. Thus, the mothers of some of the fifty-four offspring received the CPD beginning 12 days before parturition; other offspring were not given the diet until 30 days after birth. Under controlled conditions, all animals received this diet ad libitum for a period of 90 days, including the prenatal exposure. The animals whose mothers received this diet beginning 12 days prenatally were 78 days old at the time of sacrifice, while the animals beginning the diet at 30 days of age were 120 days old when sacrificed (Table I).

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‡Containing an average of 18.2 ppm of fluorine by analysis.

§Whole wheat flour, 30 per cent; corn starch, 20 per cent; confectioners' sugar, 15 per cent; whole powdered milk, 30 per cent; alfalfa, 4 per cent; and sodium chloride, 1 per cent. This contained an average of 1.4 ppm of fluorine by analysis. The brand of whole wheat used in this diet contained an average of 0.8 ppm of fluorine.

TABLE I

DENTAL CARIES EXPERIENCE OF HAMSTERS FED A CARIES-PRODUCING DIET FOR NINETY DAYS DURING VARYING PERIODS OF PRE- AND POSTNATAL LIFE

LITTER	SEX	NO.	AVERAGE						DAYS AGE CPD BEGUN
			MA	CN	AA	TS UPPER	TS LOWER	TS	
A	F	3	4.5	6.0	2.3	3.1	1.3	4.4	−12*
	M	5	8.6	11.0	20.0	40.0	10.5	50.5	
B	F	3	4.3	5.7	1.9	0.7	1.6	2.3	− 6
	M	2	4.5	4.5	1.6	1.0	0.9	1.9	
C	F	2	3.0	3.0	1.6	0.6	1.3	1.9	− 5
	M	3	2.7	2.7	1.4	0.6	0.8	1.4	
D	F	1	1.0	1.0	0.3	0.3	0.0	0.3	− 5
	M	1	0.0	0.0	0.0	0.0	0.0	0.0	
E	F	5	1.6	1.6	0.7	0.2	0.8	0.9	− 4
	M	3	1.3	1.3	0.6	0.1	0.8	0.9	
F	F	4	1.5	1.5	0.4	0.1	0.4	0.4	− 3
	M	1	2.0	2.0	2.3	0.0	6.3	6.3	
G	F	1	1.0	1.0	0.5	0.0	0.5	0.5	− 1
	M	2	3.5	4.5	1.3	0.8	0.5	1.3	
H	F	1	1.0	1.0	1.5	0.0	4.5	4.5	+ 4
	M	3	1.7	1.7	2.3	6.0	0.2	6.2	
J	F	2	8.0	10.0	7.5	11.5	1.5	13.0	+ 8
	M	1	11.0	11.0	26.0	54.5	12.3	66.8	
K	F	3	8.7	12.3	16.5	23.4	8.8	32.2	+14
	M	2	11.5	15.0	37.6	63.1	37.9	101.0	
L	F	3	11.0	15.5	18.6	24.5	10.4	34.9	+30
	M	3	10.7	13.3	52.6	78.0	64.7	142.7	

MA: Molars affected. AA: Areas affected.
CN: Number of cavities. TS: Total score.

*Since all the animals were maintained on the caries-producing diet for 90 days, the age at sacrifice equals 90, plus days of age when CPD was begun, e.g., Litter A = 78 days.

Part IB.—In the second part of this first experiment, ten female hamsters and their offspring were employed in a similar manner, with some animals receiving the caries-producing diet before delivery, others with their litters, after delivery. Forty-six animals were placed in nine groups which received the diet from −6 to +30 days. The main difference between Parts IA and IB was that in the latter 40 ppm of fluorine as sodium fluoride were added to the diet. (This figure was used on the basis of a report by Orland that Purina Laboratory Chow contained 37.9 ppm of fluorine.[7] Our own samples had not yet been analyzed.) In this case, we had hoped to render equal the fluorine contents of our stock ration and the CPD.

Part II.—In a second auxiliary experiment testing another caries-producing diet,*[8] and also serving as an adjunct in determining whether prenatal feeding is a factor in increasing caries susceptibility, four pregnant hamsters were used. Two of the animals received this diet at conception (−15 days of age of offspring). The offspring of the other two hamsters received the diet at weaning (20 and 22 days of age). After 90 days on this diet, including prenatal exposure in two of the litters, the animals were sacrificed. Therefore, those which received the diet at conception were 75 days of age at sacrifice, and the animals receiving the diet at weaning were 110 to 112 days of age (Table II).

*Corn starch, 56 per cent; confectioners' sugar, 10 per cent; whole powdered milk, 30 per cent; alfalfa, 3 per cent; and sodium chloride, 1 per cent. This diet contained an average of 1.2 ppm of fluorine by analysis.

TABLE II
CARIES EXPERIENCE RESULTING FROM PRE- AND POSTNATAL FEEDING OF A HIGH CARBOHYDRATE DIET

LITTER	SEX	NUMBER OF ANIMALS	AVERAGE SCORES	AGE (DAYS) CPD BEGUN	AGE (DAYS) AT SACRIFICE
a	F	2	87.8	−15	75
	M	2	102.1		
b	F	3	17.9	−15	75
	M	1	85.0		
c	F	3	5.3	+20	110
	M	4	1.6		
d	F	4	2.9	+22	112
	M	2	3.0		

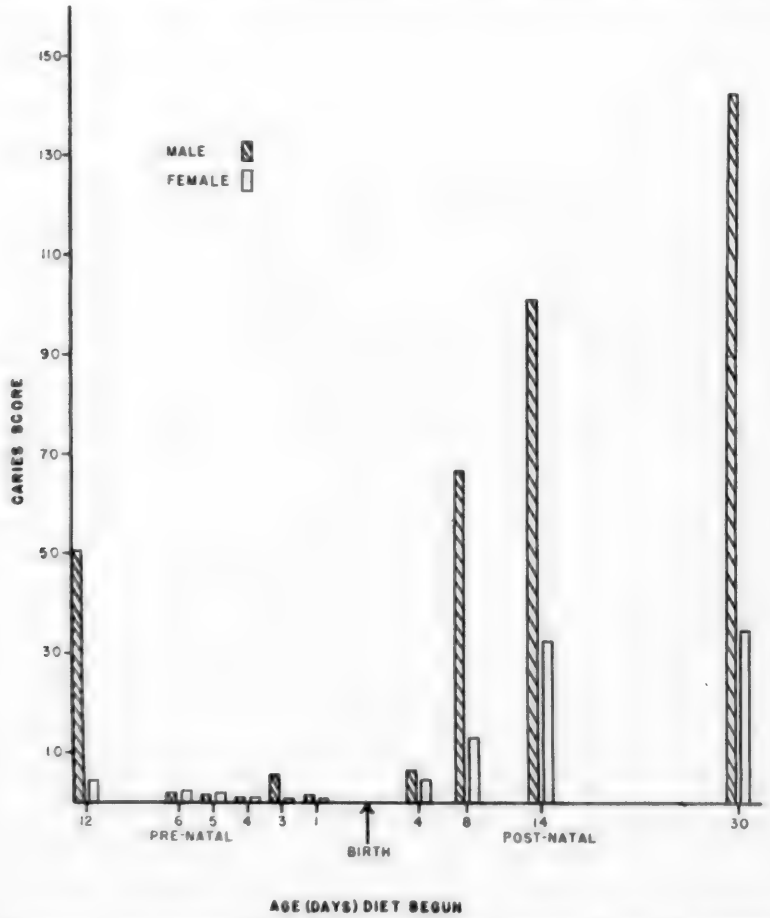

Fig. 1.—Graphic representation of caries score with the variation in age at initiation of a caries-producing diet (and the age of the animals at sacrifice).

At the indicated times, after 90 days of pre- and/or postnatal life, all the animals of both experiments were sacrificed. The jaws were fixed in formalin, dried, and stripped of soft tissue. Then they were examined under

the dissecting microscope with magnifications up to × 30, and all carious lesions were charted on special forms and scored according to the method of Keyes.[9]

RESULTS

The results of Part IA are shown in Table I. The males had more caries than the females, and the caries was more prevalent in the upper jaw than the lower. These findings are consistent with previous reports.[6, 7]

The average caries scores are seen to vary widely among the different litters. Fig. 1 shows the caries score plotted against the age in days at initiation of the CPD (which by design parallels the age of the animals at sacrifice). It appears that there was a significant amount of caries in the offspring whose mother was fed the diet beginning very early in gestation (–12 days). The caries scores then drop very low in the animals whose mothers received the CPD from 6 days before birth to 4 days after birth. Later the scores begin to increase precipitously even until initiation at 30 days of age, a time when the crowns of all teeth are formed.

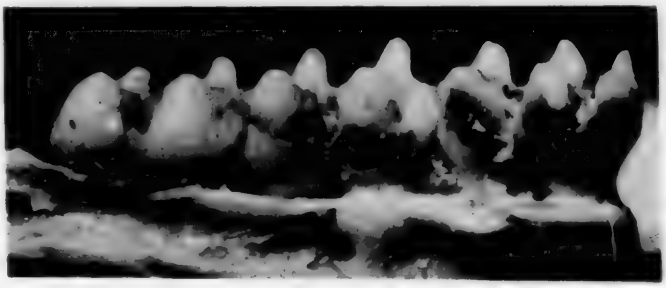

Fig. 2.—Buccal aspect of mandibular molars of a hamster which received the caries-producing diet plus fluoride. Typical enamel "fractures" are seen on the second molar. The lesion on the first molar (right) is partly artifact. Note the intact tubercle at the bottom of the buccal sulcus of the third molar.

In Part IB where the fluorinated caries-producing diet was used, only two of the forty-six animals developed carious lesions. These animals were both 23 days old at initiation of the caries-producing diet and had a combined total caries score of only 1.5. One interesting observation made in the fluorine animals was the presence of a peculiar type of enamel lesion. Since practically no caries was found in these animals, small defects of the enamel surface were discerned easily. It was soon noted that in nearly every animal of this group, enamel had been fractured off or, in some way, lost from one or more of the small ledges or tubercles found at the gingival termination of the buccal and lingual sulci of the second and third molars (Figs. 2). Such lesions which had the appearance of fractures, were seen most frequently on the buccal and lingual sulci of the second and third molars (Fig. 2). Such lesions any tooth which had formerly presented fine developmental ledges or tubercles. The dentin underlying the "fracture" was stained various shades of brown.

Subsequently, the jaws of the animals of Part IA were rechecked, and these minute lesions were found here also, though in many cases carious lesions superimposed the areas in question. In speculation, it is possible

that the coarser particles of the diet (e.g., whole wheat,) may produce such fractures during mastication, and it is not inconceivable that the exposed dentin is then more readily susceptible to the initiation of dental caries. It is not demonstrated, however, that this is a major contributory factor in the development of hamster caries.

The results of the second experiment (Part II) are shown in Table II. Here it can be seen that in the animals whose mothers received the caries-producing diet beginning at conception (litters a and b) there resulted a very high caries score, in contrast to a much lower score in the ones that did not receive this diet until weaning (litters c and d). Note that the former animals were much younger at sacrifice than the latter.

DISCUSSION

It must be remembered that uncontrollable factors were present; since all the animals were sacrificed 90 days after the initiation of the diet, this necessarily meant that the animals were of widely varying actual ages at termination of the experiment, and thus the erupted teeth were exposed to the local influence of the CPD for varying lengths of time. Although fifty-four animals were used in Part IA of the study, the eleven groups were small, and due to the nature of the experimental design, were necessarily lacking in litter-mate controls.

In the second experiment (II) the results obtained are very interesting in respect to the age factor. Since the animals with the high caries scores were only 75 days of age at sacrifice, while those with low scores were 110 to 112 days of age, these results are at variance with those of Part IA. However, the two females which received the caries-producing diet at conception delivered very small litters, and these young were all very tiny and scrawny, appearing very poorly nourished. Since it has not been conclusively shown that the general physical and nutritional condition of an organism does not play a role in the incidence of dental caries, this consideration must be weighed.

From these experiments it is difficult to conclude that any clear-cut major role is played by prenatal feeding of a high carbohydrate diet in the induction of dental caries. However, since appreciable amounts of caries occurred in those animals of Part II which were fed a caries-producing diet from the date of their conception (–15 days), and in those animals of Part IA which received a different but effective caries-producing diet from twelve days before birth, one must not overlook the possibility that a critical period may exist somewhere between –6 and –15 days, during which time such a diet may influence the incidence of dental caries in later life.

In the added fluorine experiment (IB) it had been hoped to show a gradation in the amount of caries developed depending on the time of initiation of the fluorine. However, no caries developed in any animal of this group (with the two exceptions noted), so it must be concluded that 40 ppm of fluorine, as used here, will protect the teeth of the hamster against caries if

this element is initiated at least by the time all the teeth have come into occlusion.

If the sample determinations of the fluorine content of the stock ration can be considered as representative of the whole ration, then the alleged 18.2 ppm of fluorine was not sufficient to prevent caries in these animals, even though it was fed all during gestation and through the first 30 days of life. More probably, the fluorine in this ration was not present in a biologically active state as far as caries prevention is concerned.

In this study, there is no way to measure the effect on caries of approximately 1 ppm of fluorine which was present in the two caries-producing diets, nor is the form in which the element was present known.

SUMMARY AND CONCLUSIONS

1. Animals receiving a caries-producing diet initiated at various pre- and postnatal ages developed varying degrees of caries, most marked in those initiated at 8 to 30 days after birth.

2. Animals receiving another type of caries-producing diet exhibited more caries when the diet was initiated at time of conception (–15 days) than did those initiated at weaning. The former animals were in poorer physical and nutritive condition when compared to the latter which had received a stock ration during this developmental period.

3. Animals receiving 40 ppm of fluorine as NaF in a caries-producing diet were almost completely protected against caries regardless of the ages at which the fluorine was initiated.

4. Minute fracturelike lesions of the enamel of the molars of the animals were found, and a possible relationship between these and dental caries in the hamster is discussed.

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A PROGRESS REPORT ON THE BACTERIOLOGIC ASPECT OF THE CARIES RESEARCH PROGRAM, COLLEGE OF DENTISTRY, UNIVERSITY OF CALIFORNIA

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HISTOLOGICAL studies of initial, microscopic, carious lesions of the enamel have shown that filamentous organisms predominate in the plaque on the surface, while spheroidal-appearing organisms are always present within the enamel beneath the plaque.[1, 2, 3] To determine the etiologic agent of caries, it would be desirable to isolate, by bacteriological methods, the organisms seen within such primary lesions of the enamel. This, however, presents a complicated technical problem. The hardness of the tooth structure, the minuteness of the area to be cultured, the small number of organisms within it, and, finally, the abundant opportunity for contamination to occur from the tooth's exterior surface, make a satisfactory bacteriologic examination of these lesions extremely difficult.

The following procedure has been tried as an attempt to solve the problem: Extracted human teeth were collected in sterile saline and placed in the refrigerator within a few hours after extraction. Within the next twenty-four to forty-eight hours they were examined for small carious lesions with the aid of a wide-field binocular microscope. Only those exhibiting very early carious lesions, as evidence by whitish or brownish discoloration of the enamel, were selected for the study. With a dental bur a block about 5 mm. × 5 mm. × 2 mm. in size, encompassing the lesion, was cut from each tooth. After scrubbing these blocks with soap and water, their surfaces were sterilized by immersion in formalin, "Roccal" or acetone, or by exposure to chloroform vapor. After freeing them of the disinfecting agent by rinsing, or by allowing it to evaporate. the sterility of the block surfaces was tested by incubating the blocks in nutrient broth or thioglycollate media* for at least one week. The blocks were then crushed, individually, in a modified dental swager, with aseptic precautions. The pulverized material was suspended in a small quantity of broth to be distributed into a variety of bacteriologic media. These solid and liquid media of neutral (pH 7) or acid (pH 5) reaction were incubated aerobically, anaerobically, or under partial oxygen tension for one to two weeks.

To date, eight carious lesions have been cultured in this manner. The results have been discouraging. Growth of organisms has failed to occur in most of the media inoculated. In the few instances where growth of organisms has occurred, their type and distribution have indicated that they were probable air contaminants. These results suggest that the organisms within the carious

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*Difco.

lesions are not cultivatable under the conditions used, or else that they have been destroyed by diffusion of the sterilizing agent during the surface sterilization process.

Because of the difficulties encountered in isolating the organisms within the early carious lesion of the enamel, studies on the bacterial flora of its surface plaque have been undertaken. The filamentous organisms cultured from these areas were predominantly actinomycetes.[4, 7] The ability of these actinomycetes to produce caries in unerupted hamster molars has been tested in vitro.[4] The lesions developed in vitro were remarkably similar to those which occur in hamster molars in situ.[3, 5, 6] An etiologic association between actinomycetes and caries has also been suspected by Onisi[8] in his work with dental strains. Our experiments have been continued, using other strains of actinomycetes, streptococci, and, in addition, organisms presumably of the lactobacillus and diphtheroid groups. About 175 hamster molars have been included in the study to date. Histological examination of twenty of these has now been completed.

Microscopic carieslike lesions have been produced in three hamster molars with the same actinomycete strain. Similar lesions have been produced in other molars with five other actinomycetes. The lactobacilli growing in the absence of dextrose at a neutral pH have also shown some tendency to penetrate the enamel and produce microscopic carieslike lesions. This result has been variable; it has occurred twice with two lactobacillus strains and failed to occur twice with the same two strains. Penetration failed to occur with two other lactobacillus strains. The lactobacilli growing in dextrose broth produced an acid pH of 3.5 to 4.0, in which the tooth enamel was entirely dissolved. The histopathologic picture is totally unrelated to caries.

The production of microscopic carieslike lesions by the lactobacilli at a neutral pH, in the absence of dextrose, suggests that the cariogenic properties of these bacteria may not necessarily be related to their acidogenic properties. The fact that two distinct groups of organisms—the lactobacilli and the actinomycetes—have produced carieslike lesions in these experiments suggests that the carious process may result from the action of more than one specific organism. The highly artificial conditions of these experiments must be emphasized, however. It is possible that the organisms seen penetrating the enamel are utilizing the nutrient broth which may have diffused into it rather than the organic content of the enamel matrix. To rule out the possibility of broth utilization, two further experiments are being conducted: (1) aseptically dissected hamster molars are being placed directly into washed-cell saline suspensions of the organisms, in the absence of broth, and (2) aseptically dissected hamster molars are being placed into broth cultures of bacteria not common to the oral flora to determine whether they, too, can produce carieslike lesions under these experimental conditions.

The caries in vitro experiments are also being performed on unerupted, impacted human molars. Since it is difficult to extract impacted human molars

aseptically, these molars are autoclaved after extraction. It is realized that this sterilization process may produce artifacts in the tooth structure which will confuse the histopathologic picture.

A further report of the results of these experiments will be made when the histologic examinations of the teeth have been completed.

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